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Transplant Infections

Fourth Edition

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Preface

The success of the previous editions of *Transplant Infections*, as a reference work to bring together information directed at the management of the infectious complications occurring specifically in immunocompromised individuals undergoing transplantation, has led to the creation of this fourth edition. No other text focuses solely on exogenously immunosuppressed transplant patients, and no text combines solid organ and hematopoietic stem cell transplantation (historically referred to as bone marrow transplantation). Many texts focus on immunocompromised patients, but the field of transplant infectious diseases has evolved over the past 25 years as a field unto itself, with conferences devoted solely to this specialty, and guidelines, both national and international, being developed for the management of such patients. In addition, peer-reviewed journals now exist that publish information on this specialized area, and training programs devoted to the subspecialty of transplant infectious diseases within the field of infectious disease are being developed.

The field of transplant infectious diseases has continued to grow and expand since the third edition was published in 2009. In this edition, we have continued to highlight differences between solid organ and hematopoietic stem cell transplantation by adding additional chapters where we felt this differentiation would give added value to the readers. We have added a chapter on the new rapidly evolving topic of the microbiome. The major problem of antibacterial resistance has also been addressed more in depth by having separate chapters on gram-negative and gram-positive bacterial infections. We have also expanded some chapters on viral infections, such as the respiratory viruses, since recognition of the importance of these pathogens has grown, and new diagnostic testing is now available in real time. We have added a chapter on long-term care of the transplant recipient, given the success of transplantation and the increasing number of long-term survivors. A number of new authors have been added, and chapters have been substantially revised or completely rewritten.

This edition remains a globally inclusive product of leading authors and investigators from around the world. Perspectives from Argentina, Australia, Brazil, Chile, New Zealand, Western Europe (Italy, Spain, Sweden, Austria, Germany, France, and Switzerland), the United States, Canada, and Israel have been synthesized in this edition.

We continue to believe that much can be learned from appreciating both the similarities and the differences in the pattern of infections and the resulting morbidity and mortality in various transplant settings. Our goal with this textbook is to provide background and knowledge for all practitioners who work with transplant patients, in order to improve both the care and outcomes of transplant recipients and to provide a framework for education of physicians, transplant coordinators, and trainees in the field. As success in the field continues to grow, we hope that this text will provide a knowledge base that would advance the field and make transplantation safer for all who need this lifesaving intervention. We thank all the contributors for their effort and trust the reader will find this a valuable reference text as they care for transplant recipients.

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Part I
Introduction to Transplant Infections

1 Introduction to Hematopoietic Cell Transplantation

Andrew R. Rezvani and H. Joachim Deeg

1.1 Introduction

The lymphohematopoietic system is the only organ system in mammals with the capacity for complete self-renewal. Therefore, donation of hematopoietic stem cells (HSC) does not result in a permanent loss for the donor. Reports on the therapeutic use of bone marrow to treat anemia associated with parasitic infections date back a century [1, 2], but not until the observations on irradiation effects in Hiroshima and Nagasaki and the ensuing systematic research into hematopoietic cell transplantation (HCT) in animal models were the principles of HCT established [1, 3, 4].

In 1957, the first clinical transplant attempts of the modern era were undertaken [1, 5, 6]. As predicted from animal studies, patients who underwent transplantation from allogeneic donors developed graft-vs.-host disease (GVHD) [4]. Patients transplanted from syngeneic (monozygotic twin) donors generally did not develop GVHD, but many of them died from progressive leukemia, apparently because of a lack of the allogeneic graft-vs.-leukemia (GVL) effect, which had been described by Barnes and Loutit in murine models [7]. These studies immediately established that allogeneic HCT functioned as immunotherapy.

Beginning in the late 1950s and early 1960s, Dausset et al. characterized the first histocompatibility antigens in humans [8]. Epstein et al. were the first to show the relevance of those histocompatibility antigens for the development of GVHD in an outbred species [9]. Initially, the only source of HSC used clinically was bone marrow. However, cells harvested from peripheral blood, either after recovery from chemotherapy or after the administration of hematopoietic growth factors such as granulocyte-colony stimulating factor (G-CSF), were shown to result in accelerated hematopoietic recovery after autologous transplantation. These cells, as well as cord-blood cells, are now being used with increasing frequency in allogeneic HCT [10, 11].

1.2 Rationale and Indications for Hematopoietic Cell Transplantation

Current indications for HCT are summarized in Table 1-1. The majority of HCT are performed to treat malignant diseases. Myelosuppression is the most frequent dose-limiting toxicity of cytotoxic therapy for malignancies. Infusion of HSC—autologous or allogeneic—as a "rescue" procedure allows for the dose escalation of cytotoxic therapy, until toxicity in the next most sensitive organs (intestinal tract, liver, or lungs) becomes dose limiting. This strategy, often referred to as high-dose therapy with stem cell rescue, has been used extensively in the past. However, progressive dose intensification, although possibly effective in disease eradication, has resulted in minimal, if any, improvement in survival because of increases in therapy-related toxicity and mortality. These observations, combined with an increasing appreciation of the central role of immunologic graft-vs.-tumor reactions in the success of allogeneic HCT, have led to new concepts of transplant conditioning [12].

"Replacement" therapy in patients with congenital or acquired disorders of marrow function, immunodeficiencies, or storage diseases represents a second indication for HCT. Patients with severe autoimmune diseases (e.g., rheumatoid arthritis, Crohn's disease, or systemic sclerosis) represent another group of patients who may benefit from HCT [13, 14]. In contrast to the benefit of graft-vs.-tumor alloreactivity in malignant diseases, patients with nonmalignant disorders are unlikely to derive any benefit from donor alloreactivity.

Finally, HSC (or their progeny) may be effective vehicles for gene therapy and for immunotherapy. Objectives of gene therapy include the replacement of defective or missing enzymes (e.g., adenosine deaminase, glucocerebrosidase) or of the defective gene [15, 16]. Experience with the use of allogeneic cells, often T lymphocytes, as immunologic

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Table 1-1. Categories of disease treated with hematopoietic cell transplantation[a]

Malignant
Hematologic malignancies
Acute leukemias
Chronic leukemias
Myelodysplastic syndromes
Myeloproliferative neoplasms
Non-Hodgkin lymphoma
Hodgkin lymphoma
Plasma cell dyscrasias (e.g., multiple myeloma)
Selected solid tumors
Germ cell tumors
Ewing sarcoma
Neuroblastoma
Nonmalignant
Acquired
Aplastic anemia and red cell aplasias
Paroxysmal nocturnal hemoglobinuria
Autoimmune disorders (e.g., multiple sclerosis, systemic sclerosis, rheumatoid arthritis, Crohn's disease)
Congenital
Immunodeficiency syndromes (e.g., SCID)
Hemoglobinopathies
Congenital anemias (e.g., Fanconi anemia)
Storage diseases (e.g., mucopolysaccharidoses)
Bone marrow failure syndromes (e.g., dyskeratosis congenita)
Osteopetrosis

[a]This list is not all encompassing; transplants have been carried out for various other disorders.

bullets is more extensive. Donor lymphocyte infusion (DLI) for reinduction of remission in patients with chronic myelogenous leukemia (CML) who had relapsed after HCT has been remarkably successful, leading to application of this approach in other diseases. A modification of this strategy is the use of genetically modified donor lymphocytes expressing a "suicide gene," which can be activated to abrogate adverse effects of DLI, particularly GVHD [17].

The principles of immunotherapy are also exploited prominently in allogeneic HCT following reduced-intensity conditioning (RIC), also referred to as non-myeloablative or "mini"-transplants (both terms, however, are misleading, as the end result is intended to be "ablation" of the disease, and a mini-transplant is still a full transplant, albeit with a lower-intensity conditioning regimen). In this approach, the intensity of the conditioning regimen is reduced to prevent early mortality, but enhanced donor anti-host reactivity is required to eliminate host cells (Figure 1-1).

1.3 Sources of Hematopoietic Stem Cells and Donor Selection

HSC can be obtained from a variety of donors and cellular compartments, including the bone marrow, peripheral blood, and cord blood. The choice of stem cell source is dependent upon several factors. Although autologous marrow or peripheral blood stem cells (PBSC) are theoretically available for every patient (feasibility has been reported even for patients with severe aplastic anemia), these would not be useful without genetic manipulation for genetically determined disorders and would be suboptimal for malignant disorders, because of the concern of contamination with malignant cells and the lack of an allogeneic antitumor effect. Autologous marrow or PBSC can be purged of contaminating malignant cells by chemical means or by antibodies which recognize tumor cells. However, slow engraftment and residual tumor cells that resist purging limit the usefulness of this approach. Currently available purging methods have generally failed to show clinical benefit and thus none are widely used [18, 19], although research continues into more effective approaches to HSC purification and tumor purging [20].

Generally, each full sibling has a 25 % chance of sharing the complete HLA genotype with another sibling. Somewhat less than 1 % of patients will have a syngeneic (identical twin) donor. The lack of an HLA-identical related donor in more than 70 % of patients has led to the development of large data banks of volunteer unrelated donors and research into alternative allograft sources such as HLA-haploidentical family members and umbilical cord blood.

Supported by the efforts of the National Marrow Donor Program in the United States, the Anthony Nolan Appeal in the United Kingdom, the DKMS in Germany, and other groups internationally, more than 20 million volunteer donors have been typed for HLA-A and HLA-B and a rapidly increasing number also for HLA-C, HLA-DR (DRB1), and HLA-DQ antigens. The probability of identifying a suitably HLA-matched unrelated donor for a Caucasian patient in North America is about 75 %. This probability is lower for other ethnic groups, in part because of lower representation in the data bank and in part because of greater polymorphism of the HLA genes. As a result, the likelihood of finding a fully HLA-matched unrelated donor for some ethnic minorities in the United States may be as low as 16 % [21].

Cord-blood cells, generally not matched for all HLA antigens of the patient, are being used with increasing frequency. Because of the immunologic naivete of umbilical cord-blood cells and the low T-cell content, these cells can be transplanted across significant (major) HLA barriers with acceptable rates of GVHD. Suitably HLA-matched umbilical cord-blood units can be found for 80–100 % of patients [21]. The second major alternative source of hematopoietic grafts is HLA-haploidentical family members. These donors are available for nearly all patients, since they include any parent, any child, and many siblings. With the use of posttransplant cyclophosphamide as GVHD prophylaxis, HLA-haploidentical allogeneic HCT can be performed with similar or perhaps even lower rates of acute and chronic GVHD compared to HLA-identical related or HLA-matched unrelated donors [22]. Currently, the optimal alternative-donor source of hematopoietic cells remains unclear. Contemporaneous single-arm studies of HLA-haploidentical

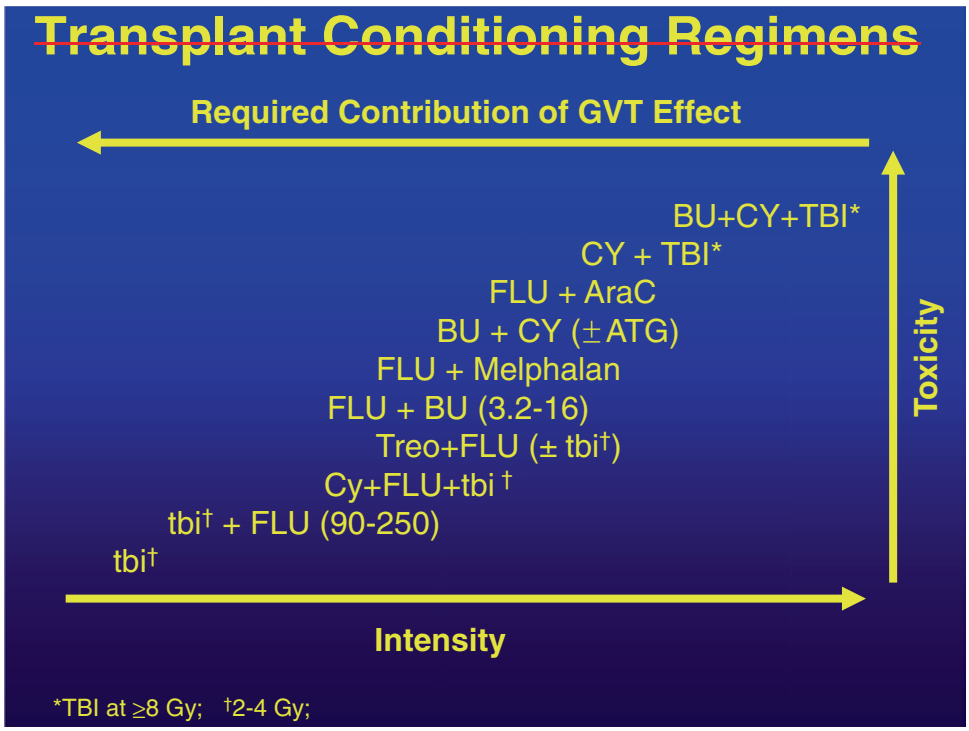

FIGURE 1-1. Selected conditioning regimens for hematopoietic cell transplantation, arranged by relative intensity, toxicity, and reliance on immunological graft-vs.-tumor effects. Abbreviations: *GVT* graft-vs.-tumor effect, *BU* busulfan, *CY* cyclophosphamide, *TBI* total body irradiation (high-dose, ≥12 Gy; low dose, 2–4.5 Gy), *FLU* fludarabine (at doses of 90–250 mg/m^2), *ATG* antithymocyte globulin, *araC* cytarabine arabinoside, *TREO* treosulfan (3×10 to 3×14 g/m^2).

vs. cord-blood transplantation suggest that overall survival is comparable, with somewhat higher rates of relapse with haploidentical donors and higher rates of transplant-related mortality with umbilical cord blood [23]. A national multicenter randomized clinical trial is currently underway in the United States, organized by the Blood and Marrow Transplant Clinical Trials Network (BMT-CTN), comparing these two sources of hematopoietic cells.

1.4 Transplant Procedure

1.4.1 Transplant Conditioning

1.4.1.1 Rationale for Conditioning

1. To ablate the patient's disease, or at least to reduce the number of malignant or abnormal cells to below detectable levels (this applies to allogeneic, syngeneic, and autologous donors). Ablation appears to allow for greater efficacy of graft-vs.-tumor reactions.
2. To suppress the patient's immunity and to prevent rejection of donor cells (this applies to allogeneic, but not to autologous, HCT). Immunosuppression is also needed in preparation for some syngeneic transplants, apparently to eliminate autoimmune reactivity which may interfere with sustained hematopoietic reconstitution.

The notion that conditioning is necessary to "generate space" in the transplant recipient has been abandoned. Donor cells, given in sufficient numbers and replete with T cells, create their own space and proceed to repopulate the recipient's marrow [24].

Exceptions to the requirement for pretransplant conditioning for allogeneic HCT exist in some children with severe combined immunodeficiency (SCID), because their underlying disease does not allow them to reject transplanted donor cells, and in patients in whom even partial donor engraftment can completely correct the genetic defect [25].

1.4.1.2 Modalities for Transplant Conditioning

Modalities used to prepare patients for HCT have been reviewed extensively elsewhere [26, 27]; a subset of commonly used regimens is shown in Figure 1-1. In principle, conditioning for HCT can include the following approaches:

1. Irradiation, in the form of total body irradiation (TBI), total lymphoid irradiation [28], or modifications thereof. Many conventional TBI regimens deliver 12–14 Gy over 3–6 days. In addition, bone-seeking isotopes (e.g., holmium) and isotopes (e.g., ^{131}I, ^{92}Y) conjugated to monoclonal antibodies (MAbs) directed at lymphoid or myeloid antigens (e.g., anti-CD20, CD45) are in use [29–31]. TBI may also be a component of RIC regimens, usually at lower doses of 2 Gy [32].
2. Chemotherapy (e.g., cyclophosphamide, 120–200 mg/kg over 2–4 days) is included in many conventional regimens. Busulfan (available in oral and intravenous formulations)

at 16 mg/kg (or lower doses), targeted to predetermined plasma levels, is often used in combination with cyclophosphamide. Other agents, including etoposide, melphalan, thiotepa, cytarabine, and, more recently, treosulfan [33, 34], may be used either alone or in combination and with or without irradiation.

3. Biologic reagents (e.g., antithymocyte globulin [ATG]) or MAbs directed at T-cell antigens or adhesion molecules suppress recipient immunity. Others are directed at antigens expressed on the recipient's malignant cells; in addition, cytokines or cytokine antagonists are being investigated. Anti-T-cell therapy predisposes the individual to viral infections, in particular CMV and the reactivation of Epstein–Barr virus (EBV) with the risk of developing EBV-related lymphoproliferative disorders (PTLD) after transplantation [35].

1.4.2 Marrow Harvest

The marrow donor receives general or regional (e.g., epidural, spinal) anesthesia, and, under sterile conditions, multiple aspirates of marrow are obtained from both posterior iliac crests. Additional potential aspiration sites are the anterior iliac crests and the sternum. Approximately 10–15 mL/kg of donor weight is collected. If no ABO incompatibility exists and if the marrow is not to be subjected to any in vitro purging procedure, the resulting cell suspension is infused intravenously without manipulation. The rate of serious or chronic complications among healthy bone marrow donors is approximately 1 % [36].

1.4.3 Alternative Stem Cell Sources

HSC circulate at low concentrations in blood [37]. Their frequency increases dramatically during the recovery phase following cytotoxic therapy and after the administration of recombinant hematopoietic growth factors such as G-CSF, which dislodge cells from the marrow. Peak blood concentrations of $CD34^+$ cells are typically reached on day 4–5 after initiating G-CSF. A single leukapheresis may be sufficient to harvest the number of HSC required for a transplant. For autologous procedures, the goal is to collect at least $2–5 \times 10^6$ $CD34^+$ cells/kg recipient weight; for allogeneic transplants, the goal is $2–11 \times 10^6$ $CD34^+$ cells/kg, although the optimum dose has not been determined and doses outside this range are sometimes used [38].

Umbilical cord blood represents a segment of the peripheral circulation of the fetus and is easily accessible after delivery. Cord-blood cells are less immunocompetent than adult cells and might therefore carry a lower risk of inducing GVHD than adult cells. The concentration of HSC in umbilical cord blood is high, but the small volume that is usually available (80–150 mL) had initially limited the use of these cells to children and smaller adults. In larger adults, approaches have included the use of two cord-blood units to ensure adequate cell dose and engraftment, as well as ex vivo expansion of hematopoietic precursors in umbilical cord-blood units for infusion together with an unmanipulated cord-blood unit [39, 40].

1.4.4 Purging

There are several reasons to purge collected donor cells or to fractionate them into subpopulations. In autologous HCT, the goal is to eliminate contaminating tumor cells, either by negative selection (removal of tumor cells with antibodies or physicochemical means) or by positive selection (purification of $CD34^+$ cells from the graft). Conversely, in allogeneic HCT, one may want to retain certain cell populations (e.g., $CD4^+$ cells) with potential for later uses such as posttransplant DLI. With the development of "tandem" HCT, combining high-dose therapy and autologous HCT to reduce the tumor burden with a subsequent allogeneic HCT to exploit the GVT effect, purging of autologous cells has lost some of its relevance.

1.4.5 Hematopoietic Stem Cell Infusion: The Actual Transplant

Donor cells are infused intravenously via an indwelling central line, often a Hickman catheter. Directed by cell surface molecules which interact with receptors on endothelial cells, HSC home to the marrow cavity. The actual infusion of stem cells is generally uneventful, though it can occasionally cause transient mild hypotension or hypersensitivity reactions, or reactions to the chemical used to cryopreserve autologous cells.

1.5 Care After Transplantation

Complications of HCT, including infections, are related to the underlying disease, the preparative regimen, and the interactions of donor cells with recipient tissue (GVHD with immunosuppression and end-organ damage). All patients experience at least transient pancytopenia, although this may be mild with RIC regimens. Patients undergoing high-dose conditioning generally develop severe pancytopenia, including neutropenia, within days after completion of conditioning. This period may last 2–4 weeks with marrow allografts, 10–14 days with mobilized PBSC grafts, or 4–6 weeks with umbilical cord-blood grafts. The period of neutropenia ends with engraftment of the donor cells, clinically defined by stable increases in the white blood cell count. Cytopenias are less pronounced after RIC, and the pattern of engraftment may be less apparent in the peripheral white blood cell count. Engraftment in these patients is generally documented by demonstrating donor chimerism by cytogenetic or molecular means in peripheral blood leukocytes and bone marrow.

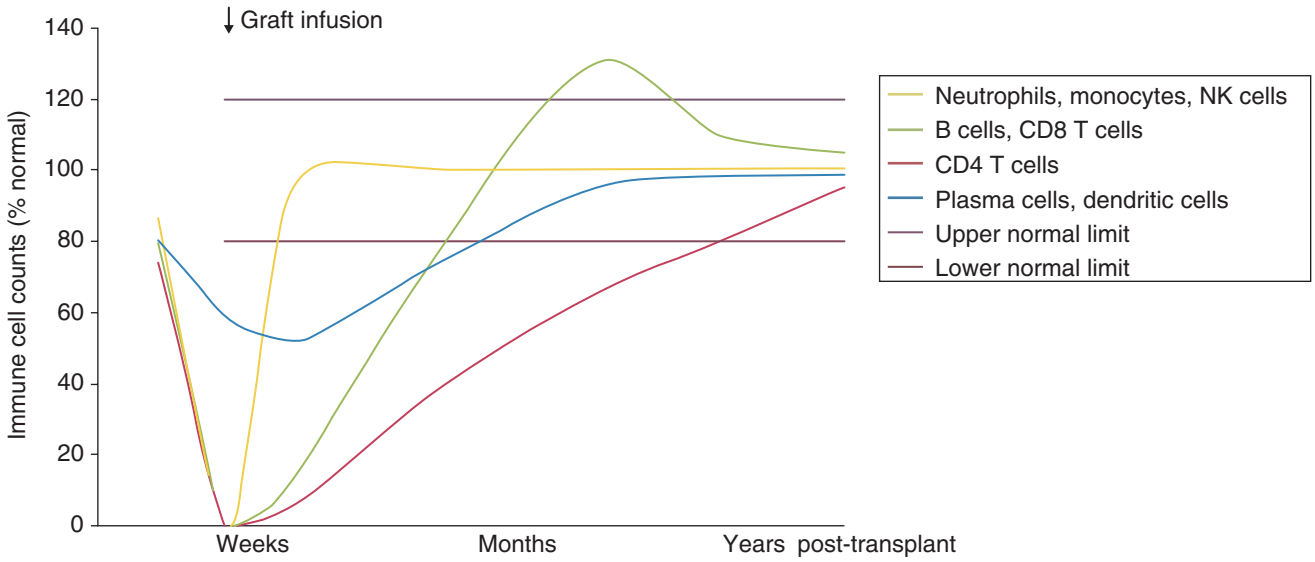

FIGURE 1-2. Approximate pattern of immune cell recovery after high-dose conditioning and hematopoietic cell transplantation. With the use of reduced-intensity conditioning, nadirs tend to be higher and to occur later. These recovery rates are influenced by clinical variables, in particular graft-vs.-host disease, stem cell source, and patient age. Storek J, Immunological reconstitution after hematopoietic cell transplantation - Its relation to the contents of the graft, Expert opinion on biological therapy, 8(5):583–97, copyright 2008, Informa Healthcare. Adapted with permission of Informa Healthcare.

Most patients prepared with high-dose regimens require transfusion support with platelets, red blood cells, or both. Transfusion requirements are substantially reduced in patients prepared with RIC regimens, because the nadir of cells often is in a range in which no transfusions are required [41]. An increasing number of transplants, both autologous and allogeneic, are being performed primarily in the outpatient clinic rather than in the hospital, due to the increased use of RIC and improvements in logistical and supportive care.

Quantitative and functional deficiencies of granulocytes and T lymphocytes for various periods after HCT are responsible for most of the infectious complications seen after HCT (Figure 1-2). While all patients receive prophylactic antimicrobials, granulocyte transfusions are not routinely given. Studies performed in the 1980s suggested that laminar air flow (LAF) rooms and gastrointestinal decontamination could reduce the frequency of infections and the duration of febrile episodes, but neither is used routinely in modern practice because of the high cost of LAF and the availability of effective broad-spectrum antibiotics [42]. It should be emphasized, however, that current research is again focused on the role of the intestinal microbiome in transplant outcomes, particularly in GVHD.

The most widely used modality of GVHD prophylaxis is the in vivo administration of immunosuppressive agents, such as methotrexate, cyclosporine (CSP), glucocorticoids, tacrolimus (FK506), mycophenolate mofetil (MMF), sirolimus, and others, either alone or in combination. At many institutions, the current standard is a combination of a calcineurin inhibitor with methotrexate or MMF, but several other combinations are used. Novel regimens have demonstrated promising results in single-arm studies, but have not yet proven superior in randomized clinical trials [43]. In one randomized trial, the addition of ATG to standard GVHD prophylaxis significantly decreased rates of acute and chronic GVHD, although it also increased the risk of fatal EBV-driven posttransplant lymphoproliferative disease and did not improve overall survival [35, 44]. Due to the non-selectivity of these agents, recipients are broadly immunosuppressed and thus susceptible to infections. In vitro T-lymphocyte depletion of donor marrow for GVHD prophylaxis may obviate the need for immunosuppressive treatment after HCT; however, the elimination of mature T cells is associated with a risk of rejection, delayed immunologic reconstitution, an increased risk of PTLD, and, for some disorders, disease recurrence. Both immunodeficiency and therapeutic immunosuppression predispose the patient to infections.

1.6 Hematologic Recovery

Patients conditioned with high-dose regimens are at risk of infections early after HCT, due to granulocytopenia until the transplanted donor cells produce new effector cells. If the donor marrow is T cell depleted, recovery of blood counts may be further delayed. If the granulocyte count at day +21 after transplantation is <200 cells/μL, patients are often given G-CSF or granulocyte-macrophage colony-stimulating factor. After PBSC transplants, engraftment (defined by >500 granulocytes/μL) occurs as early as day +9 or +10, thus

clearly shortening the length of granulocytopenia. Rather slower recovery (over several weeks) may be seen with cord-blood transplants.

One advantage of RIC regimens is the slow decline of patient cells, so that donor cells have begun to recover before patient cells have reached their nadir. Consequently, several groups have reported lower rates of early infection after RIC conditioning as compared with high-dose conditioning [45, 46].

1.7 Immunologic Recovery

All components of the innate and adaptive immune systems are deficient after HCT. Cell-mediated immunity, chemotaxis, and neutrophil function are severely impaired even after autologous transplants. The development of GVHD substantially impairs immune reconstitution, through a combination of direct graft-vs.-host toxicity to the thymus resulting in altered T-cell selection and use of immunosuppressive therapies to treat GVHD. Optimal immune reconstitution can only occur in the absence of GVHD.

1.7.1 Uncomplicated Recovery

Shortly after HCT, damaged epithelial barriers facilitate the penetration of pathogenic bacterial or fungal organisms. Mucosal surfaces begin to heal within a week or 2 of completion of high-dose conditioning, helped by the recovery of granulocytes and their scavenging function, even though phagocytosis and superoxide production may still be impaired. After the transplant, the volume and immunoglobulin content of saliva also improve with time, facilitating oropharyngeal cleansing. Even with uncomplicated recovery, T- and B-cell-mediated immune responses against viral, bacterial, fungal, and other organisms are broadly suppressed; natural killer (NK) cells recover more quickly. To some extent, the pattern of immune recovery is dependent on the immunity of the donor from whom the transplanted cells originated. The pattern of immunocompetence is also influenced by the recipient's prior antigen exposure, whether to the pathogen itself or in the form of a vaccine. Much of the literature on immune reconstitution after allogeneic HCT describes patients who were prepared with high-dose conditioning. While the use of RIC regimens has increased rapidly in recent years, there are fewer data available on immune recovery in this setting, and the impact of RIC on immune reconstitution remains somewhat unclear. Preliminary studies suggest that the tempo of immune recovery may be faster after RIC, at least by quantitative measurements [47, 48], but late immune function may be similar to that seen after high-dose conditioning [49].

1.7.2 B Cells

B-cell numbers are very low or undetectable shortly after high-dose conditioning, but may rise to supranormal levels by 1–2 years [50]. Recovery is faster with autologous than with allogeneic HCT; memory B cells lag behind naive cells. Early, but not late, recovery for both populations is faster for PBSC recipients than for marrow recipients [51]. The B-cell compartment is generally replaced completely by donor-derived cells, except in patients with T+B− SCID, in whom the recipient's B cells tend to persist [52]. Some antibodies of host origin (e.g., ABO isoagglutinins) that are derived from long-lived plasma cells may be detectable for months or even years after HCT. Persistently low B-cell counts after HCT may be associated with a high risk of infection [53]. In the era of targeted therapy, treatment with B-cell-directed MAbs such as rituximab before or after HCT may also impair and delay B-cell reconstitution [54, 55]. Some evidence suggests that poor B-cell reconstitution can lead to a dysregulated homeostatic environment with high levels of B-cell activating factor (BAFF) and other B-cell survival factors, which in turn promote the survival of alloreactive B cells and associate with an increased risk of chronic GVHD [56–58].

During recovery, fewer B cells express CD25 and CD62L; more express CD9c, CD38, IgM, and IgD; and antigen density is increased (as in neonatal B cells). $CD5^+$ cells may or may not be increased. Immunoglobulin gene usage appears to be restricted shortly after HCT and to be skewed toward the V-segments that are frequently used in neonatal B cells (e.g., VH6). Concordantly, the antibody repertoire is restricted [59]. IgG and IgA production may be abnormal for 1–2 years after HCT. Serum isotype levels after grafting recover in the same sequence as they evolve in neonates (i.e., IgM, IgG1, and IgG3 recover early, but IgG2, IgG4, and IgA may not follow until much later) [60]. Many of the early antibodies are autoantibodies, or else have irrelevant specificities. Antibodies with relevant specificities recover only if the antigen is encountered, and they recover more quickly if both patient and donor are immune. At 3 months after HCT, total IgG levels in recipients of allogeneic PBSC tend to be lower than those in marrow recipients. Antibodies to polysaccharide antigens tend to recover later than those directed at proteins. B-cell counts and IgM levels may recover more quickly after RIC as compared to high-dose conditioned patients, though IgA recovery is delayed in both groups [48]. In addition to quantitative deficits in B-cell number and immunoglobulin levels, the B-cell pool early after HCT is marked by qualitative functional impairment. Isotype switching is deficient in the absence of effective T-cell help. Additionally, B cells from transplant recipients have a decreased capacity for somatic hypermutation independent of T-cell help, suggesting an intrinsic or environmental defect. Thus, B-cell deficits after HCT are comprised of at least three factors: low B-cell numbers,

decreased T-cell help, and intrinsic defects such as impaired somatic hypermutation.

Antibody responses to vaccination are almost universally lower than those of normal controls, and repeated boosters are required. Responses are better in younger individuals and in those with T-cell-replete grafts; this may be related to CD4 recovery, which is faster in younger individuals. The policy has been to delay revaccination until 1–2 years after HCT to minimize the risk of potential side effects and to increase the probability of antibody responses.

1.7.3 T Cells

1.7.3.1 CD4⁺ Cells

The number of $CD4^+$ T cells is low for 1–3 months after high-dose conditioning and rises slowly toward normal over several years. The kinetics are similar following both autologous and allogeneic transplants. Early in the process, most cells are memory T cells; naive T cells follow gradually, particularly in older patients. These kinetics might be related to diminished thymic function, but data on thymic recovery after HCT in older patients are conflicting [61, 62]. After PBSC transplantation, both naive and memory $CD4^+$ T cells are more abundant than after marrow transplantations. Early after HCT, most $CD4^+$ T cells are derived from transplanted mature T cells and T-cell precursors; later, they are stem cell derived, at least in pediatric patients. $CD4^+$ T-cell reconstitution may occur more rapidly after RIC than after high-dose conditioning [48].

$CD4^+$ T cells generally express CD11a, CD29, CD45RO, and HLA-DR and less CD28, CD45RA, and CD62L, consistent with the prominence of memory cells. Responses to polyclonal stimuli are low. Proliferative responses to frequently encountered antigens (e.g., Candida species) tend to normalize over 1–5 years, whereas responses to unlikely antigens (e.g., tetanus) remain subnormal. Responses to neoantigens (e.g., dinitrochlorobenzene) and recall antigens (e.g., mumps) are abnormally low for 2–3 years after HCT.

1.7.3.2 CD8⁺ Cells

$CD8^+$ T cells are low for 2–3 months after HCT; subsequently, they rise quickly, resulting in an inversion of the typical CD4:CD8 ratio. These $CD8^+$ cells are largely memory cells expressing CD11a, CD11b, CD29, CD57, HLA-DR, and CD45RO but little CD28, CD45RA, and CD62L. The presence of a $CD11b^+CD57^+CD28^-$ phenotype suggests anergic or suppressor-type CD8 cells. $CD8^+$ cells appear to be derived from transplanted T cells and stem cells.

CMV-specific or EBV-specific $CD8^+$ cells can be transferred successfully from donor to recipient, and they may persist for at least 18 months in the absence of glucocorticoid treatment [63, 64]. Even established and refractory CMV infections can be treated effectively by the infusion of expanded CMV-specific $CD8^+$ donor cells; this approach has been most effective in the absence of glucocorticoid treatment for GVHD or in the setting of T-cell-depleted allotransplantation [65]. The logistical difficulty of generating CMV-specific cells for clinical use has been a barrier to the wide application of this approach. However, several groups have reported progress in developing simpler and more scalable means of producing virus-specific donor T cells for infusion [66, 67].

The role of immunoregulatory $CD4^+CD25^+$ T cells (T_{reg}) in clinical transplantation remains to be fully established. Adoptive transfer of T_{reg} has been explored as a treatment for GVHD [68], but its efficacy remains to be confirmed.

1.7.4 Antigen-Presenting Cells

Monocytes reach normal levels within 1 month after high-dose conditioning, although their function may remain impaired for a year [69]. G-CSF-mobilized PBSC contain large numbers of monocytes with altered cytokine profiles that may suppress allogeneic T-cell responses. G-CSF-mobilized monocytes settle in tissues in the early posttransplantation period.

The reconstitution of dendritic cells (DC), their maturation, and the development of DC1 and DC2 have been incompletely characterized. DC precursors in the blood recover within 6 months, and DC reconstitution appears to be a clinically important event. Low numbers of DC at 1 month after RIC and allogeneic HCT have been associated with an increased risk of mortality and disease relapse; $CD16^+$ DC counts at 3 months also had strong prognostic significance [70]. In one study, low numbers of plasmacytoid DC at 3 months after HCT were associated with higher risks of infection and death [71]. Langerhans cell levels are low in the early posttransplantation period, but return to normal by 6 months. Follicular DC are reconstituted rather slowly, which may contribute to the delayed return to function of the germinal centers and memory B cells.

1.7.5 Natural Killer Cells

NK cells recover rapidly after HCT. With the recognition of the killer inhibitory receptor, there has been renewed interest in these cells because of their possible function in engraftment and the prevention of relapse [72]. Robust NK-cell reconstitution after allogeneic HCT has been associated with reduced relapse incidence and with improved survival [73]. Recent data suggest that NK cells may also play a role in controlling CMV reactivation after allogeneic HCT. Some studies have found that CMV viremia produces a rapid expansion of adaptive NK cells, leading to protection against CMV disease [74, 75]. However, other groups have reported no correlation between the numbers of $NKG2C^+$ NK cells and protection from CMV viremia [76, 77].

1.8 Graft-vs.-Host Disease and Graft-vs.-Leukemia Effects

Acute and chronic GVHD occur in 10–50 % and 20–50 %, respectively, of patients after HLA-identical sibling HCT and in 20–60 % and 30–70 %, respectively, of patients who undergo transplantation from alternative donors. Without prophylaxis, virtually all recipients of allogeneic transplants develop GVHD. Acute GVHD may occur within days (e.g., among HLA-nonidentical recipients) or by 3–5 weeks after HLA-identical transplantation following high-dose conditioning. The main target organs are the immune system, skin, liver, and intestinal tract. Risk factors for acute GVHD include donor/recipient HLA mismatches, the use of unrelated rather than related donors, and TBI-containing conditioning regimens [78]. Importantly, after RIC, classic manifestations of acute GVHD may develop several months after HCT and may overlap considerably with those of chronic GVHD [79]. Features of chronic GVHD may be present as early as 50–60 days after HCT. Therefore, revised diagnostic criteria distinguish acute from chronic GVHD on the basis of pathology and biology, rather than time of onset [79]. Patients with late-onset or recurrent acute GVHD, and those with overlap syndromes of acute and chronic GVHD, have particularly poor prognoses. A recent analysis of data from the Center for International Blood and Marrow Transplant Research (CIBMTR) indicates that the incidence of chronic GVHD has been steadily increasing over time [80], possibly associated with the increasing use of alternative donors and G-CSF-mobilized peripheral blood rather than bone marrow allografts. On the other hand, there are data suggesting that posttransplant cyclophosphamide, given as GVHD prophylaxis, reduces the incidence of chronic GVHD.

HCT is a unique form of allotransplantation in that many recipients develop donor/host tolerance over time, to the point that maintenance immunosuppression can be completely discontinued. In patients without GVHD, all immunosuppressive medication is generally stopped by 6 months to 1 year after HCT. Even chronic GVHD is not necessarily a lifelong condition; many patients with chronic GVHD develop tolerance and resolution of GVHD over time and are able to discontinue immunosuppressive treatment. A study from the Fred Hutchinson Cancer Research Center indicated that, on average, immunosuppressive treatment was required for approximately 2 years in patients with chronic GVHD [81].

The immunopathophysiology of GVHD is complex. The initial damage to host tissue is induced by the transplant-conditioning regimen [82]. The subsequent development of acute GVHD involves antigen presentation. Shlomchik et al. showed that host DC play a pivotal role in this process [83]. Interactions of MHC antigens (with bound peptides derived from minor histocompatibility antigens) and T-cell receptors lead to activation, clonal expansion, and differentiation of donor T cells. Accessory T-cell surface molecules, such as CD4 or CD8, also contribute to the immunologic synapsis between T cells and antigen-presenting cells. The effector phase leads to host cell destruction via inflammatory signals, cytolytic effects, and programmed cell death (apoptosis). Inflammatory cytokines, which are primarily released from the gut, allow the transfer of endotoxins and lipopolysaccharides (LPS) into the circulation, triggering macrophage activation. The result is the further production of cytokines, such as tumor necrosis factor α (TNFα) and IL-1 [84], leading to target cell death and the expression of costimulatory molecules, such as CD80, CD86, and MHC class II antigens, on DC; T-cell stimulation; and the release of T helper-1 (Th1) cytokines (IL-2, interferon-a [IFN-a]).

Recent experiments also emphasize the role of other cytokines, particularly TNFα, IL-15, and IL-18 [85]. In mouse models, TNFα is a central mediator of GVHD that works predominantly in the intestinal tract. Anti-TNF antibodies prevent or ameliorate GVHD in mice [86]. In humans, anti-TNF therapy appears active in treating established acute GVHD [87], but ineffective as GVHD prophylaxis [88]. However, the actions of different cytokines and effector cells (e.g., large granular lymphocytes) and regulatory T cells (T_{reg}) are still incompletely understood. The role of T_{reg} with a $CD25^+CD4^+$ phenotype, which is functionally reminiscent of the classic "suppressor T cell," is still being defined, as is the role of Th17 cells [89–91].

Elevated serum levels of soluble Fas ligand have been observed in some patients with GVHD [92]. Fas-mediated apoptosis may also be involved in the control of alloreactive T cells [93]. Perforin-mediated cytotoxicity also plays a role in both GVHD and GVL effects [94, 95]. However, even T cells from mice doubly deficient in both FasL and perforin can cause GVHD after mismatched-donor HCT [96].

1.8.1 Stem Cell Source

The kinetics of GVHD depend upon the source of stem cells. PBSC mobilized by means of chemotherapy or G-CSF have been used extensively for allogeneic and autologous stem cell rescue, and they are associated with rapid hematopoietic reconstitution. G-CSF may polarize donor cells toward Th2 cells and promote regulatory T-cell function, favoring the development of tolerance [97]. Several clinical studies suggest that the incidence of acute GVHD is similar in marrow and PBSC recipients, whereas the incidence of chronic GVHD appears to be increased with PBSC [98]. However, the higher incidence of chronic GVHD with PBSC may not be associated with a significant increase in mortality. In fact, the results of several trials suggest that, particularly in patients with high-risk malignancies, survival is improved in PBSC recipients, perhaps due to a more vigorous GVT effect

or to more rapid immune reconstitution [99, 100]. Bone marrow allografts are often preferred in nonmalignant diseases, where GVT effects are unnecessary and minimization of GVHD is the overriding goal.

Studies directed at the mechanisms involved in the effects of PBSC show an increased production of IL-10, decreased levels of TNFα in monocytes from G-CSF-mobilized PBSC, and reduced expression of costimulatory molecules and MHC class II antigens. Thus, a tolerogenic effect related to monocytes may be present, possibly juxtaposed with a countereffect due to increased numbers of T cells.

Chronic GVHD has prominent features in common with autoimmune disorders [101], including the presence of T lymphocytes with abnormal cytokine profiles (e.g., secretion of IL-4 and IFNγ). Thymic damage, inflicted by the conditioning regimen as well as by preceding acute GVHD, leads to the failure of intrathymic selection and an escape of autoreactive cells to the periphery [102]. A similar mechanism appears to be responsible for a syndrome analogous to allogeneic GVHD which can develop after syngeneic or autologous HCT. Recently, the role of B cells in chronic GVHD has attracted renewed attention, based in part on reports indicating that the anti-CD20 MAb rituximab could treat or prevent chronic GVHD [58]. Some groups have posited that autoantibodies produced by autoreactive B-cell clones contribute directly to chronic GVHD [103]. Alternately, B cells may contribute indirectly, by influencing effector and regulatory T-cell compartments, as they do in other autoimmune diseases [104, 105].

As stated, the immune system is a major target of GVHD. Immunodeficiency is a key feature of GVHD that is amplified by the immunosuppressive therapies used to treat GVHD, thereby rendering patients highly susceptible to infections. The risk is further accentuated by damage to various barrier structures, particularly the skin and intestinal tract. All aspects of immune recovery after HCT are impaired or delayed in patients with GVHD; in patients with chronic GVHD, immunoincompetence may extend over years.

1.8.2 Prevention and Treatment of GVHD

Methods of GVHD prophylaxis are summarized in Table 1-2. Combination regimens of methotrexate (or MMF) and a calcineurin inhibitor (cyclosporine or tacrolimus) are the most widely used regimens after high-dose conditioning. The addition of prednisone to methotrexate and CSP has been shown to increase or to decrease the incidence of acute GVHD, depending on the timing of prednisone administration [106, 107]. Some trials of FK506 (tacrolimus) combined with methotrexate have shown an incidence of GVHD lower than that observed with CSP; however, disease-free survival was not improved [108]. Other trials have suggested that tacrolimus may be superior to CSP only in the unrelated-donor setting [109]. Some groups have explored the use of sirolimus in addition to or in place of methotrexate, although this approach did not prove superior to standard tacrolimus/methotrexate in a randomized clinical trial and was possibly associated with a higher risk of chronic GVHD [43]. Various prophylactic regimens have been employed after RIC HCT, including combinations of MMF with CSP, sirolimus, and T-cell depletion.

TABLE 1-2. Modalities of graft-vs.-host disease prevention

Selection of histocompatible donors
T-cell depletion
Ex vivo
Negative selection and removal of T cells
Removal of naïve T cells
Positive selection and purification of hematopoietic stem cells
In vivo
Anti-T-cell monoclonal antibodies (e.g., alemtuzumab)
Posttransplant cyclophosphamide
Pharmacologic inhibition of T-cell function
Calcineurin inhibitors, antimetabolites, etc.
Cytokine blockade
Gnotobiosis

T-cell depletion of donor marrow, which is clearly effective in reducing the incidence of GVHD, has increased the probability of graft failure, posttransplant infection, and relapse of leukemia [110, 111]. Graft failure problems have been overcome in part by the in vivo administration of a Campath-1H MAb or of polyclonal ATG [35, 112, 113]. Alternatively, additional DLI may be given preemptively or therapeutically after HCT to reduce the risk of disease recurrence and graft failure. Some preliminary studies have suggested that depleting specific donor T-cell subsets from the DLI product may render this approach more effective [114, 115].

Much of the morbidity and mortality associated with acute and chronic GVHD derives from immunosuppression and the associated risk of potentially lethal infections. If GVHD develops despite prophylaxis, aggressive therapy is required. Glucocorticoids remain the first-line therapy for acute and chronic GVHD. Despite decades of investigation, there is no standard, effective second-line therapy for steroid-refractory acute or chronic GVHD [116]. Steroid-refractory GVHD poses a significant challenge from the standpoint of infectious disease, as patients are typically treated with escalating doses of various immunosuppressive agents in an effort to control alloreactivity. Patients with severe or refractory GVHD are profoundly immunosuppressed, and infection remains one of the major causes of mortality in this population. Standards of care for infection surveillance and prevention vary institutionally in patients receiving treatment for active GVHD, but in general such patients require close monitoring for opportunistic infections, both typical (e.g., CMV, respiratory viruses) and atypical.

1.9 Graft Failure

Graft failure (either primary or secondary) occurs in <5 % of patients undergoing allogeneic HCT. Major risk factors for primary graft failure after high-dose conditioning include myeloproliferative neoplasms, the use of marrow rather than PBSC as a stem cell source, HLA mismatch, ABO incompatibility, and cryopreservation of the graft before infusion [117]. Several host cell types, particularly $CD8^+$ T lymphocytes and NK cells, participate in the rejection of donor cells. Donor T cells counteract this host response, thereby facilitating engraftment and preventing rejection. As a consequence, T-cell-depleted marrow is more susceptible to rejection, as described above. Graft failure is associated with a very high risk of infections due to prolonged neutropenia. Patients with graft failure after high-dose conditioning generally will not have spontaneous complete autologous hematopoietic recovery and require salvage with a second HCT if clinically feasible [118]. In contrast, RIC regimens generally allow recovery of host hematopoiesis if the donor graft is rejected.

1.10 Delayed Complications

By 2 years after HCT, about 80 % of patients have returned to pretransplant activities. However, some patients develop delayed or chronic complications (Table 1-3). These complications are related to elements of the conditioning regimen (most importantly, irradiation or high-dose busulfan), side effects of HCT (chronic GVHD, immunodeficiency), or combinations thereof. The presence and severity of chronic GVHD are the dominant factors influencing quality of life in long-term survivors of allogeneic HCT [119]. Life-threatening complications include infections, pulmonary dysfunction, autoimmune disease, musculoskeletal problems, and new malignancies.

Table 1-3. Delayed complications of hematopoietic cell transplantation

Chronic graft-vs.-host disease
Infection
Airway and pulmonary disease (e.g., bronchiolitis obliterans)
Autoimmune dysfunction
Impaired growth and development
Endocrine dysfunction
Sterility
Cataracts
Dental problems
Osteopenia or osteoporosis
Aseptic necrosis of the bone
New malignancies
Psychosocial dysfunction

1.11 Summary

HCT offers effective and potentially curative therapy for many life-threatening malignant and nonmalignant diseases. Side effects include acute toxicity to multiple organs, the development of GVHD and immunoincompetence, and secondary effects related to immunosuppressive therapy. The result is a high susceptibility to infections, which are a major cause of morbidity and mortality after HCT. Both GVHD and its therapy are important predisposing factors; thus, reducing the incidence and severity of GVHD is an important component of efforts to reduce posttransplant infections. Transplant-conditioning regimens have undergone multiple modifications aimed at reducing early toxicity and permitting HCT in older patients and those with medical comorbidities. RIC may allow for faster immune recovery and better infection control and has expanded the availability of HCT to higher-risk patient populations. New antibiotics and methods of cellular therapy have also enhanced the ability to eradicate infections. Recent advances in laboratory techniques have facilitated a greater understanding of the role of the host microbiome in immune function. Host-microbiota interactions may be particularly important in the setting of allogeneic HCT, as the sites most heavily colonized with microbiota are also primary targets for GVHD (e.g., skin and gut). The interplay between the donor immune system and the host microbiome may play a significant role in modulating alloreactivity, tolerance, and GVHD. Early work has associated specific posttransplant changes in the gut microbiome with higher or lower risks of GVHD [120, 121], although more study is required to disentangle causation and confounding factors, such as antibiotic use, which may influence the microbiome. The emerging understanding of the role of the microbiome in transplantation has been reviewed elsewhere recently [122] and is discussed in more detail in Chap. 52.

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2
Introduction to Solid Organ Transplantation

Nagaraju Sarabu and Donald E. Hricik

2.1 Historical Perspective

Organ transplantation has been the subject of ancient myths dating back to the twelfth century. The modern era of transplantation began in the early 1900s with the development of surgical techniques for constructing vascular anastomoses [1] leading to successful kidney transplantation in dogs in 1902 [2]. The first series of human kidney transplants were performed in the Ukraine beginning in 1933, but each of five attempted transplants failed [3, 4]. Around the same time, Kuss et al. [5], Servelle et al. [6], and Dubost et al. [7] reported technically successful transplantation of kidney allografts in humans, placing the organs heterotopically in the iliac fossa, similar to the technique used in the modern-day operation. However, all of these grafts failed over a short period of time. In 1954, Murray et al. [8, 9] performed a kidney transplant between identical twins and achieved long-term function. During the subsequent 10 years, more than 30 kidney transplants between identical twins were performed worldwide.

These early transplants between identical twins were successful because the donors and recipients were syngeneic, sharing the same immune system and thus eliminating the possibility of immunologically mediated rejection of the graft. In the 1940s, the seminal experiments of Medawar first delineated the immunologic basis for allograft rejection [10] and the need for immunosuppressive therapy to achieve successful transplantation using non-syngeneic grafts. By 1963, the first human liver transplantation was performed, using early forms of immunosuppression [11]. One year later, Barnard [12] performed the first successful human heart transplant. Shortly thereafter, techniques were developed for clinical heart–lung [13] and pancreas [14] transplantation.

Since those early days, remarkable strides have been made to increase the success of organ transplantation to prolong the lives of patients with end-stage organ disease. General advances in medical science, including improvements in surgical techniques and the development of effective antimicrobial agents, have undoubtedly played a role in this success story. However, the current success of organ transplantation has been related more directly to an improved understanding of the mechanisms of allograft rejection and the development of immunosuppressive drugs capable of preventing or treating rejection.

Although transplantation offers a survival advantage and improved quality of life for most patients with end-stage organ disease, the continued disparity between the supply of allografts from deceased donors and the growing demand for these organs represents the main limiting factor in field of transplantation today. In addition, while the mechanisms and treatments for acute forms of allograft rejection are well understood, our understanding of chronic forms of rejection remains limited, and organs continue to be lost from both immune and nonimmune causes. The remainder of this chapter will review the known mechanisms of allograft rejection, the drugs used to prevent and treat rejection, and current outcomes of organ transplant recipients.

2.2 Mechanisms of Allograft Rejection

Alloimmune reactions resulting in rejection of an allograft remain the major barrier to long-term survival of transplanted organs. Immunologic tolerance can be achieved with relative ease in small animals. However, the human immune system is complex, containing redundant pathways that make tolerance difficult to achieve. Thus, in the current era, allograft rejection remains the major threat to long-term survival of transplanted kidneys and the vast majority of transplant recipients require life-long treatment with immunosuppression drugs. Delineation of mechanisms leading to allograft rejection has been critical to the development of agents capable of preventing or treating rejection.

Mammalian immune responses evolved to protect the host from infectious pathogens and to provide discrimination of

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self from nonself. An efficient response requires the recognition of pathogens and subsequent activation of key cells and soluble mediators of immunity [15, 16]. Similarly, immune responses resulting in the recognition and destruction of an allograft require cells with an ability to migrate, antigen-presenting cells (APCs), soluble mediators such as cytokines and effector cells that injure the graft.

2.2.1 Allorecognition

The major histocompatibility complex (MHC) is a set of cell surface molecules encoded by genes contained on chromosome 6 [17, 18]. The primary immunologic function of MHC gene products is to present fragments of foreign proteins, forming complexes that can be recognized by T lymphocytes through their antigen-specific receptors. Antigen presentation begins when an MHC complex binds a peptide antigen. MHC molecules are composed of a highly polymorphic polypeptide alpha chain and a monomorphic beta chain, consisting of beta2-microglobulin in the case of class I MHC. Allospecificity of class I MHC molecules, expressed constitutively on all nucleated cells, resides in the alpha chain, a polypeptide with a prominent groove or pocket that is the site where foreign proteins bind for presentation to T cells. Class II MHC molecules are expressed constitutively only on APCs, including macrophages, dendritic cells, and B cells. Adjacent portions of the highly variable alpha chain and a non-variable beta chain form a peptide groove. Highly variable amino acid residues located in the groove determine the specificity of T cell antigen recognition. The same T cell receptor (TCR) can recognize either class I or class II MHC molecules, but restrictions are imposed by the engagement of the T cell surface molecule, CD4, to class II molecules and CD8 to class I molecules. Thus, CD4-positive T cells primarily engage peptides presented by class II MHC, while CD8-positive T cells engage peptides presented by class I MHC (see Figure 2-1a).

Immediately following vascularization of an allograft, donor antigens enter the systemic circulation via APCs and travel to the spleen and lymph nodes where naïve T cells are activated. At the same time, recipient cells enter the allograft. *Direct allorecognition* occurs either in the secondary lymphoid system or in the graft. In the lymphoid system, this occurs when the recipient's naïve lymphocytes are engaged with donor APCs that have traveled to the lymph nodes or spleen. In the graft, direct allorecognition occurs when donor APCs engage with recipient lymphocytes [19]. *Indirect allorecognition* occurs in the secondary lymphatic system when donor proteins or peptides are first processed by recipient APCs and presented to the TCR by the recipient's MHC on the surface of the APC (Figure 2-1b). In the graft, indirect allorecognition occurs when recipient APCs process donor peptides and engage recipient lymphocytes by presenting those processed peptides in the groove of the recipient MHC [19]. The direct pathway of allorecognition plays a dominant role in early T cell-mediated acute rejection episodes while the indirect pathway is believed to be more important in mediating chronic rejection.

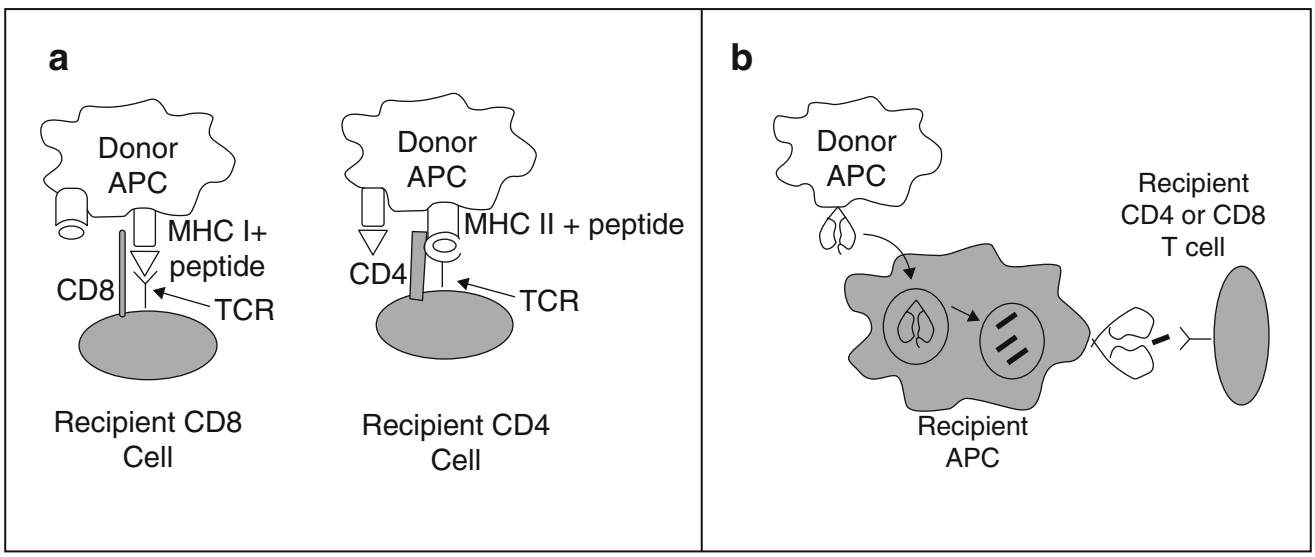

FIGURE 2-1. (**a**) Depiction of *direct allorecognition* in which a donor antigen-presenting cell (APC) presents peptide to the T cell receptor (TCR) within the context of donor major histocompatibility complex (MHC) molecule. *Left side*: presentation of a peptide within a class I MHC molecule to a CD8-positive T cell. *Right side*: presentation of a peptide within a class II MHC molecule to a CD4-positive T cell. (**b**) Depiction of *indirect allorecognition* in which an antigen is first processed by a recipient antigen-presenting cell (APC) and then presented within the context of a recipient major histocompatibility complex (MHC) molecule to either a CD4- or CD8-positive T cell. Reprinted from Am J Kidney Dis, 65(6), Donald E. Hricik, pp. 956–66, Copyright 2015, with permission from Elsevier.

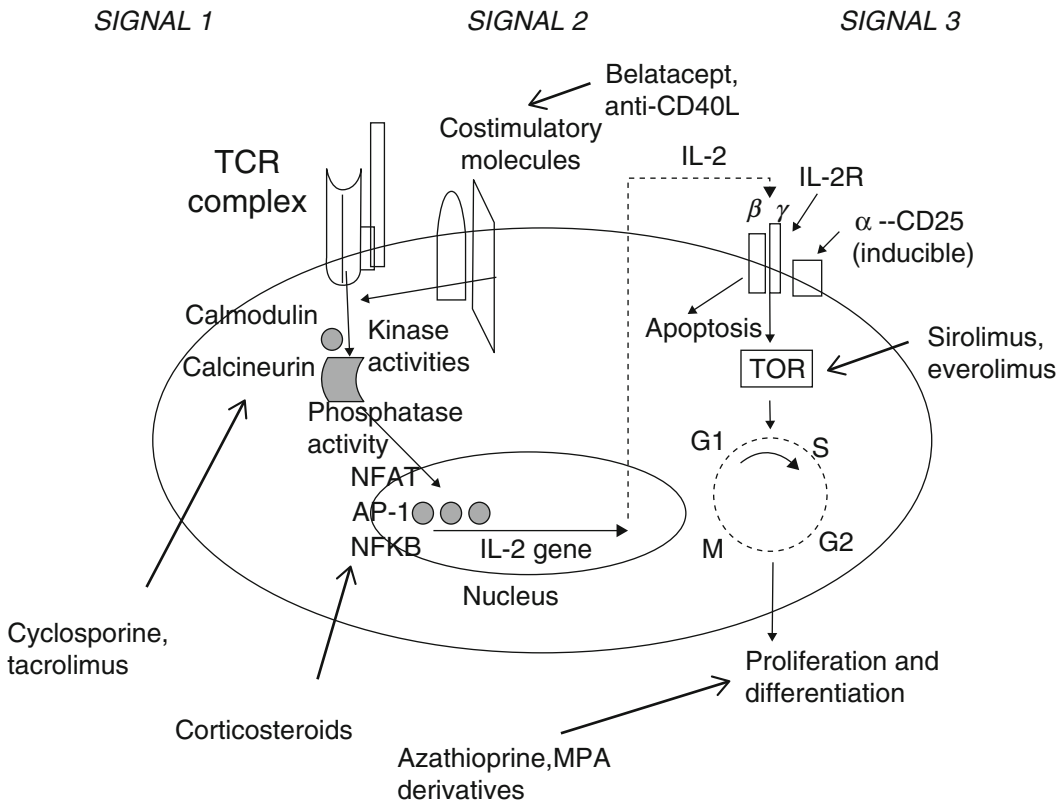

FIGURE 2-2. Schematic diagram of the three signals required for full activation and proliferation of T cells. Also shown are the sites of action of the major classes of maintenance immunosuppressive drugs. See text for details. Reprinted from Am J Kidney Dis, 65(6), Donald E. Hricik, pp. 956–66, Copyright 2015, with permission from Elsevier.

2.2.2 T Cell Activation and Differentiation

The TCR consists of two polypeptide chains, alpha and beta, that are linked to each other. The TCR is linked to another group of cell surface molecules known as CD3, a complex that consists of several covalently bound peptide chains. When the TCR binds to an MHC-presented antigen, there is a conformational change in CD3 that activates intracellular signal pathways, including tyrosine kinases located on the intracytoplasmic tails of the CD3 peptides as well as on the CD4 and CD8 accessory molecules. This antigen-driven signal, transduced by the TCR–CD3 complex to the T cell cytoplasm, has been called "signal one" (see Figure 2-2). It is essential but not sufficient alone for full activation of T cells.

A second antigen-independent signal ("signal 2"), provided through additional accessory molecules resulting in "co-stimulation" of the T cell, is necessary for full activation of the T cell [20] (see Figure 2-2). Although the family of known co-stimulatory ligands is large, the two most important are ligands between the T cell surface molecules, B28 and CD154 (CD40 ligand), and the APC surface molecules B7 and CD40, respectively. Without co-stimulation, the provision of signals through the TCR alone leads to clonal and antigen-specific anergy. The T cell does not produce cytokines and does not divide, but instead becomes unresponsive to appropriate stimulation or undergoes apoptosis.

With adequate co-stimulation, T cell activation continues, and signals are transduced to the nucleus. Phosphorylation of tyrosine residues on several proteins occurs as an immediate consequence of TCR activation. The immediate effect is the appearance of newly phosphorylated tyrosine residues on a number of proteins, leading to the generation of the second messengers such as inositol 1,4,5-triphosphate (IP3) that stimulates the release of ionized calcium from intracellular stores. Released calcium interacts with the calcium-dependent regulatory protein, calmodulin. These calcium–calmodulin complexes activate other kinases and phosphatases. One of these is calcineurin, a phosphatase that plays a key role in the activation of factors required for IL-2 gene transcription.

The transcription of IL-2 and other cytokines ultimately drive cell cycle progression ("signal 3") with help from a series of kinases, including those that act in the target-of-rapamycin (TOR) pathway (see Figure 2-2). The final results of activation are the proliferation of CD4-positive helper T cells and the maturation of CD8-positive cytotoxic T cells. Activated T cells ultimately differentiate into a number of other phenotypes including memory cells that can respond

quickly and robustly to the initially presented antigen many years after the initial presentation, and regulatory T cells that can suppress immune responses and promote tolerance.

2.2.3 Effector Mechanisms

The mammalian immune system can be divided into innate and adaptive components. *Innate immunity* is mediated by several nonpolymorphic proteins (e.g., defensins, cytokines, toll-like receptors, and complement) and cells (e.g., macrophages, dendritic cells, natural killer cells, and neutrophils) that immediately contain and eliminate infectious agents. There has been recent interest in the concept that these innate responses may interact with alloimmune mechanisms, forming a potential link between nonspecific injury (e.g., ischemia reperfusion injury or infections) and allograft rejection.

In contrast, T cells and B cells provide finely tuned specificity mediated by highly polymorphic receptors and antigen-induced clonal expansion. This *adaptive immunity* develops only days to weeks after antigen exposure. The complement system serves as an interface between innate and adaptive immunity [21]. The terminal components of complement are important effectors of graft destruction, leading to membrane injury, neutrophil infiltration, and damage to epithelial and endothelial cells. However, the complement system also is involved in T and B cell stimulation.

The Fas/Fas ligand (FasL) pathway is an important effector mechanism leading to destruction of an allograft. Fas is expressed ubiquitously on parenchymal cells, while FasL is induced upon activation of CD4-positive T cells. Cross-linking of Fas with FasL leads to activation of caspase 8 and propagation of a death signal that culminates in apoptosis.

The activation of caspase enzymes leading to irreversible cell injury with DNA fragmentation can occur independently of cell surface receptors. In addition, CD8-positive T cells express cytotoxic molecules that are lethal to cells. One of these, granzyme B, gains access to the cell by a pore structure created by perforin, another product of the CD8-positive cytotoxic T cell. Entry of granzyme B into the target cell cytoplasm ultimately leads to target cell death through apoptosis. Natural killer cells are effector cells that also produce perforin and granzyme B. In addition, they produce interferon gamma, thus promoting inflammation.

2.2.4 Role of B Cells

With the help of T cells, bone marrow-derived B cells can differentiate into plasma cells that ultimately produce antibodies specific for the original peptide antigen presented to the T cell. Several growth factors required for this differentiation have been identified recently and may ultimately serve as therapeutic targets. Mature B cells are found mainly in lymphoid follicles, in bone marrow, and in low numbers in the circulation. Differentiated plasma cells generate antibodies that can act by fixing complement or by opsonizing cells that are then killed by cell-mediated lympholysis. As noted above, B cells also serve as excellent APCs.

Recently, alloantibodies have been identified as major effectors of both acute and chronic graft injury. Alloantibodies are primarily directed against HLA antigens. However, a number of less common alloantibodies to non-HLA antigens (e.g., endothelial or epithelial antigens) have been identified and occasionally cause graft injury. Preformed antibodies to HLA antigens most commonly occur in patients who have had previous transplants, blood transfusions, or pregnancy. Less commonly, they develop cross-reactively after exposure to vaccines, viruses, or other pathogens. Preformed anti-HLA antibodies are measured by a variety of cross-matching techniques. Mixing recipient serum with the cells or HLA antigens of a specific donor performs a donor-specific cross-match. When the serum of a potential transplant recipient is "cross-matched" with cells from a large panel of potential donors, the test is referred to as a panel of reactive antibodies (PRA). Patients with high PRA (i.e., preformed anti-HLA antibodies against a large number of potential donors) are said to be "sensitized" and generally exhibit graft outcomes that are inferior to non-sensitized patients. In theory, only donor-specific antibodies (DSAs) are responsible for graft injury [22]. Transplantation is usually avoided in patients with pre-existing DSAs. However, very low titers may escape detection by even the most sensitive of cross-matching techniques. Moreover, de novo DSAs develop in as many as 15 % of kidney transplant recipients during the first posttransplant year, increase in frequency with the passage of time, and are now recognized as a major cause of late graft injury and graft loss.

2.3 Types of Allograft Rejection

Allograft rejection can be classified based on clinicopathologic criteria into hyperacute, acute, and chronic forms. However, the pathologic findings obviously vary from one organ to another. This is especially true of chronic rejection which, for example, is manifested in kidney transplant recipients as some combination of interstitial fibrosis, tubular atrophy, and/or transplant glomerulopathy, in heart transplant recipients as coronary vasculopathy, and in lung transplant recipients as bronchiolitis obliterans. Lung transplantation is unique in that chronic rejection can be defined histologically but is most often diagnosed by functional parameters such as changes in forced expiratory velocity (FEV) over time. Hyperacute rejection occurs in recipients with high titers of preformed DSAs and is a rare occurrence in the era of modern, highly sensitive cross-matching techniques.

A complete description of the pathology of acute and chronic rejection in each organ is beyond the scope of this review. Pathologic scoring systems for acute rejection have been best developed in kidney [23, 24], heart [25] transplantation. For kidney transplants, use of the Banff criteria for grading rejection has become the standard of practice [23, 24]. Most centers prefer to obtain a biopsy of the organ to facilitate treatment decisions in patients with suspected rejection, although some centers do not routinely perform pancreas transplant biopsies, mostly due to concerns about bleeding. Acute forms of rejection are usually divided into cellular and humoral types, but there are sometimes components of both cellular and antibody-mediated damage in a single tissue specimen.

Cases of acute cellular rejection that are deemed to be clinically or histologically mild are often treated initially with large "pulse" doses of corticosteroids. Patients who do not respond to pulse steroid therapy and those with clinically or histologically severe rejection are treated with antilymphocyte preparations. Algorithms for treating acute antibody-mediated rejection are less well established and vary widely from center to center. Therapeutic strategies have been best defined in kidney and heart transplantation. Traditional antilymphocyte antibodies are often employed to treat antibody-mediated rejection, based on the concern for simultaneous cellular rejection. However, treatment with plasmapheresis, anti-CD20 antibodies (i.e., rituximab), and/or IVIg is now commonly used as either primary or adjunctive therapy for humoral rejection. Chapter 3 contains a more detailed discussion of drug therapy for treatment of acute rejection.

2.4 Immunosuppressive Therapy

In this section, we will focus on the mechanisms of action of commonly used classes of immunosuppressive agents, based on our understanding of how they inhibit alloimmune responses detailed in the previous section. Chapter 3 includes a more complete discussion of clinical use of these drugs.

2.4.1 Antibodies Used for Induction Therapy

In the USA, available T cell-depleting antibodies include two polyclonal agents generated in either rabbits (rabbit antithymocyte globulin, Thymoglobulin®) or horses (ATGAM®) [26]. Rabbit ATG is currently the most popular polyclonal antibody used in the USA. However, it is technically prescribed off-label for induction therapy, being approved by the Food and Drug Administration (FDA) only for the *treatment* of acute rejection. The exact mechanisms accounting for the effectiveness of rabbit ATG (or ATGAM®) are not entirely understood. These preparations includes antibodies against numerous T cell markers including CD2, CD3, CD4, CD8, CD11a, CD18, CD25, CD44, CD45, HLA-DR, and HLA class I heavy chains. Treatment is generally associated with profound lymphopenia. The agent is effective in suppressing the cellular immune responses against a variety of antigenic stimuli, but may be less reliable in preventing antibody-mediated acute rejection. Alemtuzumab (Campath®) is an anti-CD52 humanized monoclonal antibody that binds to all T and B lymphocytes as well as most macrophages, monocytes, and natural killer cells. It is FDA approved only for the treatment of lymphoma and is used off-label for induction therapy in transplant recipients [27]. The agent causes significant leukopenia, probably via antibody-mediated lysis of lymphocytes, resulting in T cell depletion that lasts much longer than that observed with the polyclonal agents (often detectable for more than 1 year).

The only nondepleting antibody available in the USA currently is basiliximab (Simulect®) [26]. This chimeric monoclonal antibody is directed against the α chain of the interleukin-2 (IL-2) receptor (also known as CD25). Binding to this receptor inhibits the proliferative signals normally mediated by IL-2 (see Figure 2-2) without causing profound depletion of lymphocytes.

2.4.2 Maintenance Immunosuppression

Corticosteroids have multiple effects on alloimmune pathways [28–30]. These agents alter the distribution of lymphocytes, leading to their sequestration in the reticuloendothelial system. They also inhibit the proliferation and function of lymphocytes by blocking the expression of various cytokines. In addition, corticosteroids inhibit transcription factors such as activating protein-1 (AP-1) and nuclear factor-κB. As a consequence, these agents inhibit the production of IL-1 (a primary stimulus for helper T cell activation) and IL-6 (a major inducer of B cell activation), thus inhibiting both the cellular and humoral arms of the alloimmune response.

Calcineurin inhibitors include cyclosporine, a small cyclic polypeptide of fungal origin and tacrolimus, a macrolide antibiotic compound [31, 32]. Multiple formulations and generic version of these drugs are now available. Within the cytoplasm of the lymphocyte, cyclosporine binds to cyclophilin, while tacrolimus binds to FK-binding protein (FKBP). Both the cyclosporine-cyclophilin and tacrolimus-FKBP compounds bind to and inhibit calcineurin, preventing its normal function and thereby blocking T cell activation (see Figure 2-2). Thus the two agents are similarly efficacious in preventing rejection. However, they exert considerably different side effect profiles (see Chap. 3).

Antiproliferative agents include azathioprine and various derivatives of mycophenolic acid (MPA), including the original agent, mycophenolate mofetil, a prodrug that is metabolized to MPA. Azathioprine is a metabolite of 6-mercaptopurine. It is processed into purine analogs that inhibit both the de novo and salvage pathways of purine

synthesis. This inhibits the synthesis of RNA and DNA, thus blocking gene replication and cell proliferation [33]. MPA (derived either from mycophenolate mofetil or available as enteric-coated mycophenolate sodium) is a reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH), a rate-limiting enzyme in the synthesis of purines [34]. Like azathioprine, it works by inhibiting nucleic acid synthesis. However, the effect is relatively selective for lymphocytes because IMPDH plays a preeminent role in the de novo pathway for purine synthesis and lymphocytes do not have an effective salvage pathway that is present in most other rapidly dividing cells.

TOR inhibitors include sirolimus and everolimus [35, 36]. These drugs bind to FKBP in the cytoplasm but have no effects on calcineurin and instead inhibit TOR, an important regulatory kinase that normally mediates cell cycle progression (see Figure 2-2). Inhibition of TOR affects both lymphocytes and mesenchymal cells. The TOR pathway also mediates angiogenic effects so that TOR inhibitors exhibit unique anti-angiogenic properties.

Belatacept is currently the only available *co-stimulation blocker.* The drug is fusion protein that blocks T cell co-stimulation ("signal 2") mediated by the B7-CD28 ligand described above. As described in Chap. 3, the agent was developed largely as a replacement for calcineurin inhibitors [37].

2.5 Current Outcomes in Solid Organ Transplantation

This section will focus on the characteristics, outcomes, and long-term morbidities of solid organ transplant recipients in the United States. Most of the data comes directly from the 2013 Annual Data Report of the Organ Procurement and Transplantation Network (OPTN)/Scientific Registry of Transplant Recipients (SRTR) [38].

2.5.1 Kidney Transplantation [39]

The most common current causes of end-stage renal disease resulting in the need for kidney transplantation are diabetes mellitus (29.3 %), hypertension (21.8 %), and glomerulonephritis (18.3 %). Since 2002, the number of candidates on the deceased donor waiting list almost doubled from approximately 50,000 to 96,000 in 2013. In 2013, 16,901 kidney transplants were performed in the USA (11,448 from deceased donors and 5433 from living donors). By comparison, 15,197 transplants were performed in 2003. Despite this modest overall growth in transplant volume, living donation rates decreased almost 40 % during the same decade. Paired and other unrelated donations have increased since 2007, but not enough to compensate for the general decline in living donation. During the past decade, the number of recipients aged 50 years or older has increased. The use of donors after cardiac death (DCD) increased from approximately 4 % of all deceased donors in 2003 to more than 15 % of deceased donors in 2013.

Table 2-1. Donor characteristics used in calculating the kidney donor profile index (KDPI)

- Age
- Height
- Weight
- Ethnicity
- History of hypertension
- History of diabetes mellitus
- Stroke as the cause of death
- Serum creatinine
- Presence or absence of hepatitis C
- Type of donor: brain dead versus donor after cardiac death

Until recently, allocation of kidneys from deceased donors was prioritized using a point system, with points awarded for several variables including time on the waiting list, prior organ donation, HLA matching, and sensitization based on calculated PRA levels of >80 % [40]. The allocation system was revised in December 2014. In the new system, deceased donors will be scored on a cumulative percentage scale of 0–100 % using a kidney donor profile index (KDPI) based on ten donor characteristics shown in Table 2-1 [41]. The best 20 % of kidneys (KDPI of 0–20 %) are now preferentially allocated to the best 20 % of candidates based on estimated posttransplant survival and thus will virtually always be offered to candidates under the age of 50 years. The influence of KDPI scores on 1- and 2-year allograft survival rates is depicted in Figure 2-3. More priority will be given to sensitized patients in the new system. In addition, for patients who started dialysis before being approved for wait listing, waiting time will start at the time that dialysis was initiated. The impact of these new changes in the allocation system will be scrutinized heavily in the next few years.

During the past decade, death-censored graft survival for both deceased and living donor kidney recipients steadily increased at 1, 5, and 10 years. Death-censored graft survival at 90 days posttransplant is now approximately 97 % for deceased donors and 99 % for living donors. For patients transplanted between 2007 and 2011, the cumulative 24-month incidence of a first acute rejection episode was approximately 14 % for deceased donor recipients and approximately 12 % for living donor recipients.

Trends in the major components of immunosuppression protocols since 2003 have been characterized by a steady increase in the use of T cell-depleting antibodies for induction therapy, use of tacrolimus as the preferred calcineurin inhibitor, and use of mycophenolate derivatives in favor of TOR inhibitors (see Figure 2-4). Approximately 35 % of patients are not taking corticosteroids 1 year after transplantations but the SRTR data suggests that the use of steroid-free regimens has not changed appreciably since 2007.

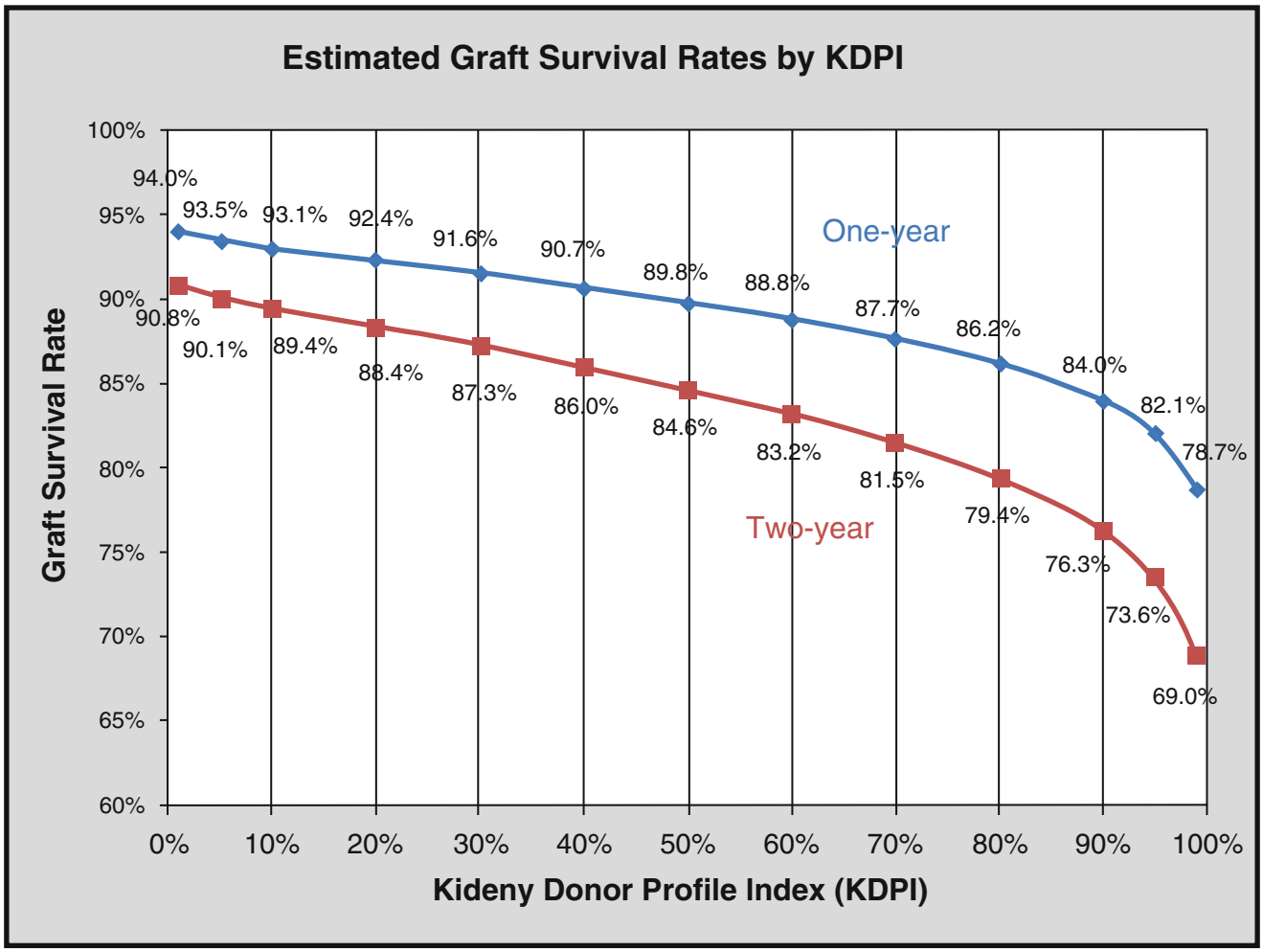

FIGURE 2-3. Influence of kidney donor profile index (KDPI) scores on 1- and 2-year graft survival rates in kidney transplant recipients. Kidney Transplantation Committee, Organ Procurement and Transplantation Network, "Proposal to Substantially Revise The National Kidney Allocation System," http://optn.transplant.hrsa.gov/PublicComment/pubcommentpropsub_311.pdf. Accessed October 30, 2015.

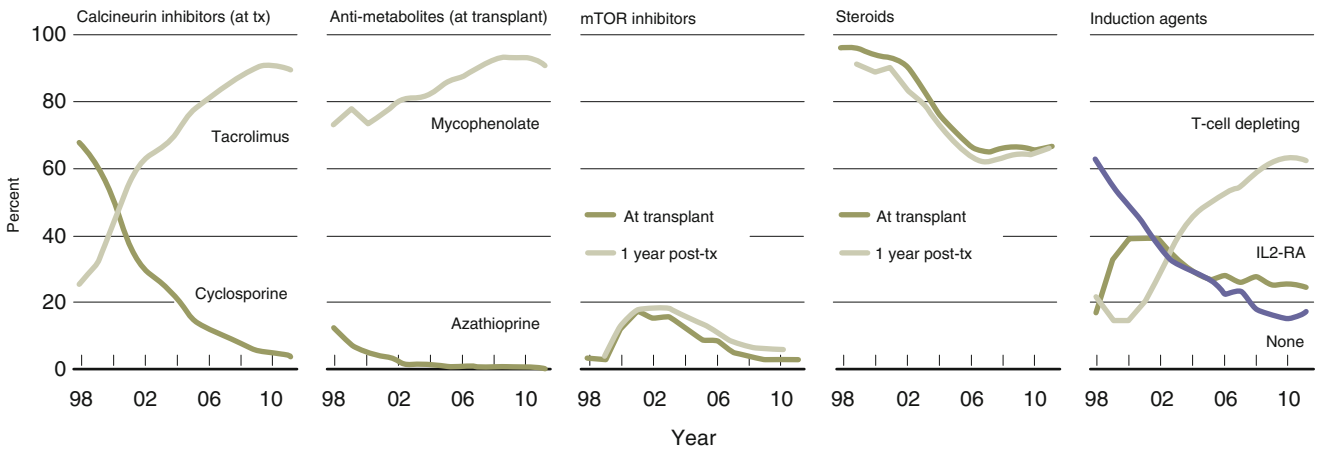

FIGURE 2-4. Trends in the use of immunosuppressant drug classes between 1998 and 2011 at the time of hospital discharge from the transplant operation (*dark lines*) and at 1 year post transplant (*light lines*). United States Renal Data System. 2014 USRDS annual data report: Epidemiology of kidney disease in the United States. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2014. The data reported here have been supplied by the United States Renal Data System (USRDS). The interpretation and reporting of these data are the responsibility of the author(s) and in no way should be seen as an official policy or interpretation of the US government.

The incidence of new onset diabetes mellitus during the first year after kidney transplant has decreased from approximately 12 % in 2005 to 5 % in 2013. By 5 years posttransplant, 0.6 % of adult transplant recipients have developed posttransplant lymphoproliferative disease. Renal function at 1 year has improved steadily. Currently, almost half of patients with functioning allografts at 6 months have an estimated glomerular filtration rate of 60 mL/min/1.73 m^2 or higher.

2.5.2 Liver Transplantation [42, 43]

Currently, the most common diseases resulting in the need for liver transplantation are hepatitis C (25 %), malignancy (usually hepatocellular carcinoma, 19.4 %), and alcoholic cirrhosis (18.4 %). The recent availability of safe and highly effective antiviral drugs capable of treating and eradicating hepatitis C will likely change this pattern in the future. In 2013, 5921 adult liver transplants were performed in the USA, including 211 from living donors. At the end of that year, just over 15,000 candidates were registered on the waiting list for live transplants. Waitlist mortality and morbidity remain problematic in liver transplantation. In 2013, 1767 patients died while waiting for a transplant and another 1223 were removed from the list being deemed too ill to undergo the procedure. Allocation of livers continues to be driven by use of the model for end-stage liver disease (MELD) scores, using a system that assigns livers to candidates with the most advanced disease [43, 44]. The MELD score is currently based on measurements of serum creatinine, serum bilirubin, and the international normalized ratio (INR) (see Table 2-2).

The proportion of liver transplant patients receiving a simultaneous liver and kidney transplant rose from 6.7 % in 2010 to 8.1 % in 2013. This proportion may decrease over time as a consequence of the Share 35 policy that went into effect in the USA in 2013. That policy requires regional sharing of livers to candidates with MELD scores equal to or greater than 35. In the first several months after instituting the policy median waiting time for such patients fell dramatically from 14 months to 1.4 months [43]. The shorter waiting times may reduce the need for simultaneous kidney transplant by decreasing the frequency of prolonged hepatorenal syndrome.

By mid-2013, 59,500 US liver transplant recipients were alive with functioning grafts. Since 1991, 1-year graft survival has steadily improved from approximately 74 % to approximately 90 % in the most recent cohort. The use of antibodies for induction therapy in liver recipients has increased only slightly in the past decade. More than 70 % of liver transplant recipients receive no induction therapy at all. Tacrolimus and mycophenolate derivatives are the most commonly used maintenance agents. Steroid withdrawal is more common after liver than after kidney transplantation. Only 40 % of liver transplant patients remain on corticosteroids 1 year after transplantation. Recurrence of hepatitis C remains a problem and accounts for graft survival being poorest among the subset of liver transplant recipients with this underlying disease. Again, the recent introduction of newer antiviral agents promises to change these statistics in the next several years.

Table 2-2. Calculation of the model for end-stage liver disease (MELD) score

- MELD score = $0.957 \times \log_e$ (serum creatinine, mg/dL)[a] + $0.378 \times \log_e$ (serum bilirubin, mg/dL) + $1.120 \times \log_e$ (INR) + 0.643
- Multiply score by 10 and round to nearest whole number. Laboratory values <1.0 are set to 1.0

[a]The maximum serum creatinine allowed in the MELD equation is 4.0 mg/dL. For patients on dialysis, the serum creatinine is automatically entered as 4.0 mg/dL

2.5.3 Pancreas Transplantation [45]

Pancreas transplantation is indicated primarily for patients with type I diabetes mellitus, but also for selected type 2 diabetics who are not obese, and who have relatively low insulin requirements. Virtually all pancreas transplants are recovered from deceased donors. Most commonly, pancreas transplantation is performed together with a kidney transplant in diabetic patients with end-stage renal failure (simultaneous pancreas and kidney, SPK) and less commonly is performed alone (pancreas transplant alone, PTA) or after a previous kidney transplant (pancreas after kidney, PAK) [46]. The major indication for a PTA is hypoglycemic unawareness.

The total number of pancreas transplants performed in United States has steadily decreased in the last decade. The reasons for this decline are not clear but possibly reflect relatively high rates of technical failure, surgical complications from the procedure [47], or improved outcomes with medical therapy alone for this special population. Just under 1500 total pancreas transplants were performed in 2002, dropping to just over 1000 transplants in 2013. The decline in volume has been more pronounced for SPK and PAK transplants than for PTA transplants. Historically PTAs were performed less commonly than SPKs or PAKs. Interestingly, in 2013, transplant rates for PAK and PAT were virtually equivalent but only about 100 transplants were performed in each of those categories. The allocation of pancreas transplants has traditionally been subject to regional variances. Current efforts are UNOS are aimed at creating a national pancreas allocation system in which candidates for SPK, PAK, or PTA will combine to form a single match run list [45]. If implemented, this system would assure that SPK candidates will not have to compete against nondiabetic kidney transplant candidates.

Immunosuppressive practice for recipients of pancreas transplants has changed little in the past 5 years. T cell-depleting induction was used in approximately 80 % of all transplants in 2013. For maintenance, tacrolimus was used in

approximately 92 % and mycophenolate in 90 % of recipients. Steroids were used in 65 % initially and in 75 % of recipients at 1 year post transplant.

Due to lack of a uniform definition for pancreas transplant failure (variably defined as re-initiation of insulin, initiation of oral hypoglycemic medications, or undetectable C-peptide), the outcomes of pancreas transplant graft survival are not as standardized as those for kidney graft failure. With this limitation, graft failure rates within the first 3 months posttransplant (often described as technical losses) have decreased steadily over the past decade from 12.4 % in 2002–2003 to 7.6 % in 2012–2013. Rates were lowest among SPK recipients (2.5 % for kidney, 4.9 % for pancreas) and comparable for PTA and PAK (10.4 % and 9.9 %, respectively). Unadjusted actual 1- and 5-year pancreas graft survival for the transplants performed in 2008 were 74.3 and 50.6 % for PTA, 85.8 and 74.3 % for SPK, and 78.7 and 62.0 % for PAK. It has been postulated that better graft survival for SPK compared to PAK and PTA is due to a relatively low incidence of rejection in this group and/or earlier recognition and treatment of rejection. This may reflect the presence of the kidney transplant, which is more amenable for a percutaneous biopsy than a pancreas transplant, and can be used as a surrogate marker for rejection in the pancreas.

The incidence of first acute pancreas rejection at 1 and 2 years was 22.1 and 27.8 % for PTA, 16.0 and 20.4 % for SPK, and 17.4 and 22.5 % for PAK. Overall incidence of posttransplant lymphoproliferative disorder at 5 years was 2.6 % for PTA, 1.0 % for SPK, and 0.9 % for PAK, and the incidence was higher among recipients negative for EBV (6.4 % for PTA and 3.6 % for SPK). The number of patients living with a functioning pancreas transplant has doubled between 2002 and 2013 from approximately 7000 to 14,000.

2.5.4 Heart Transplantation [48]

About 2500 heart transplants were performed in 2013, compared to 2100 in 2002. Cardiomyopathy is the most common indication for heart transplant, followed by coronary artery disease. The number of patients waiting for heart transplant steadily increased from 2800 in 2002 to 3200 in 2013. The waiting time for heart transplant overall has not changed significantly within this time period. In 2003, 14.8 % of candidates spent 5 or more years on the waiting list, compared with only 5.4 % in 2013. Heart transplants are allocated based on a UNOS scoring system (see Table 2-3). The proportion of candidates maintained on ventricular assist devices (VADs) at the time of wait listing increased dramatically, from 7.5 % in 2003 to 27.4 % in 2013. Because of steady improvements in VAD technology, some patients are maintained on these devices for long periods of time, either as a bridge or even as an alternative to transplantation [49].

More than half of heart transplants in the USA are performed without any induction agents, and the remainder are done with either IL-2 blocking- or T cell-depleting antibodies. More than 90 % of the patients are on a combination of tacrolimus, MPA derivatives, and corticosteroids. One-, 3-, and 5-year survival rates in patients who underwent heart transplant between 2006 and 2008 were 88.1 %, 81.3 %, and 75.3 %, respectively. Survival was slightly lower for recipients with prior VADs than for those without VADs. The number of heart transplant survivors continued to increase over time with 27,120 heart transplant recipients being alive with a functioning graft in 2013.

Table 2-3. Heart transplant candidate listing status

UNOS waiting list status (in order of priority)	Patient/management description
1A	(a) Mechanical circulatory support, excepting VADs,[a] but including an artificial heart, extracorporeal membrane oxygenator, or intra-aortic balloon pump
	(b) Mechanical circulatory support within LVAD or RVAD, with complications
	(c) Continuous mechanical ventilation
	(d) Continuous infusion of high-dose intravenous inotropic agent with continuous invasive hemodynamic monitoring
1B	(a) RVAD and/or LVAD, uncomplicated
	(b) Continuous infusion of intravenous inotropic agent
2	Awaiting heart transplant but not meeting 1A or 1B criteria
7	Temporarily unsuitable to undergo transplantation (i.e., HOLD status)

[a]*VAD* ventricular assist device, *L* left, *R* right.

Rejection remains an important cause of morbidity after heart transplant with a current cumulative incidence of acute rejection at 1 year of 23.6 %. Rejection may be recognized more frequently in heart transplantation than in other organ transplants owing to the common practice of performing serial protocol biopsies, especially in the first posttransplant year. Cytomegalovirus (CMV) infection has been strongly linked to cardiac allograft vasculopathy [50]. The leading causes of death during year 1 posttransplant are infection, cardiovascular/cerebrovascular disease, and graft failure. After year 1, however, cardiovascular/cerebrovascular disease becomes the more common cause of death, followed by infection and graft failure.

2.5.5 Lung Transplantation [51]

Lung transplantation is being performed increasingly for critically ill patients with end-stage lung disease. Allocation of lungs is based on the lung allocation score (LAS), a scoring system introduced in 2005 [52]. Pulmonary diagnoses are categorized into to four groups for the calculation of LAS: group A, obstructive lung disease; group B, pulmonary

Table 2-4. Factors used in calculating the lung allocation score (LAS)

• Underlying cause of lung disease
• Age of recipient
• Body mass index
• Presence or absence of diabetes mellitus
• New York Heart Association functional status (I, II, III)
• Forced vital capacity (FEV) (percent predicted)
• Pulmonary arterial systolic pressure
• Supplemental oxygen required at rest (L/min)
• Pulmonary capillary wedge pressure
• Distance walked within 6 min
• Need for mechanical ventilation
• Serum creatinine concentration
• pCO_2

vascular disease; group C, cystic fibrosis and immunodeficiency disorders; and group D, restrictive lung disease. The LAS system was designed to estimate waitlist mortality in a fashion that allows transplantation for compromised patients while avoiding candidates whose likelihood of survival is poor. Clinical variables used to calculate the LAS score are shown in Table 2-4. A raw allocation score is calculated based on these variables and then normalized to obtain the actual LAS, which has a range of 0–100. Higher scores indicate that the patient is more likely to benefit from a lung transplant.

In 2013, 1946 lung transplants were performed, including adult and pediatric recipients, the most ever in a single year. Bilateral lung transplantation remains the preferred procedure, accounting for approximately 70 % of lung transplants performed in 2013. In 2013, 28.7 % of all US lung recipients were aged 65 years or older, compared with 7.2 % in 2003.

Short-term survival (30-day and 1-year) and long-term survival (3-year and 5-year) have plateaued since implementation of the LAS. Overall, 5-year unadjusted patient survival was 53.6 %. Survival was consistently lowest among recipients aged 65 years or older, those with LAS greater than 60, and those in diagnosis group B. Fifty percent of lung transplants currently are performed without any induction antibody therapy. Tacrolimus and mycophenolate derivatives are the preferred agents for maintenance immunosuppression and are being used in more than 90 % of lung recipients. Almost all patients are on steroids at 1 year post transplant. About 20 % and 40 % of the patients experience first acute rejection by 12 and 24 months post transplant, respectively. About 2 % of patients develop PTLD by 5 years of posttransplant with incidence up to 6 % for patients who are serologically negative for EBV at the time of transplantation.

2.5.6 Intestinal Transplantation [53]

Improvement in the medical and surgical treatment of patients with intestinal failure has resulted in a recent decrease in the number of intestinal transplantations being performed in the USA. Short-gut syndrome remains to be the most common indication. More than half the transplants are actually combined intestine-liver transplants. The number of intestine transplants decreased from 91 in 2009 to 51 in 2013. The number of intestine-liver transplants steadily decreased from a peak of 135 in 2007 to a low of 44 in 2012, but increased slightly to 58 in 2013.

Graft survival for intestine transplants has improved over the past decade. Graft failure in the first 90 days posttransplant occurred in 14.1 % of intestine recipients and in 11.2 % of intestine-liver recipients in 2013. The graft failure rate was 24.5 % at 1 year for transplants performed between 2011 and 2012, 43.6 % at 3 years for transplants performed between 2009 and 2010, 48.5 % at 5 years for those performed between 2007 and 2008, and 68.4 % at 10 years for transplants performed between 2001 and 2002.

For induction therapy in 2013, 54 % of intestine transplant recipients received T cell-depleting agents, 11 % received IL-2 receptor antagonists, and 38 % received no induction. The initial immunosuppression agents used most commonly in 2013 were tacrolimus (95.0 %), steroids (73.0 %), mycophenolate (35.0 %), and mammalian TOR inhibitors (15.0 %). Steroids were used in 70.0 % of recipients at 1 year posttransplant. Acute rejection occurred in 35–40 % of patients at 12 months and in approximately 50 % at 24 months.

For patients who underwent intestine transplantation between 2001 and 2011, 9.9 % of intestine recipients and 6.8 % of intestine-liver recipients developed PTLD within 5 years posttransplant. The incidence was highest among recipients who were negative for EBV: 12.5 % of EBV-negative intestine recipients and 8.2 of EBV-negative intestine-liver recipients.

2.6 The Future of Solid Organ Transplantation: Strategies for Achieving Tolerance

A long-standing goal in the field of solid organ transplantation is to induce immunologic tolerance to the graft such that the host's immune system can respond normally to immune stimuli without immunosuppression and with the specific absence of a detrimental immune response directed at the transplanted organ. Studies in animal models suggest that tolerance to an allograft can be achieved under a variety of conditions including elimination of the donor-reactive immune cells (deletion), induction of immunologic ignorance (the immune system fails to recognize transplant antigens), induction of anergy, or active inhibition by regulatory T cells [54]. True immunologic tolerance has been achieved in human kidney transplant recipients when a bone marrow transplant has been performed between HLA identical donors, followed by a kidney transplant using the same

donor. Based on these experiments of nature several groups have attempted to use bone marrow ablation, either marrow or stem cell transplantation, and adjunctive combinations of early immunosuppression in an effort to achieve at least "operational" tolerance [55–57].

Tregs suppress immune responses, potentially via local cytokine production and through prevention of dendritic cell activation. The recent recognition of multiple Treg phenotypes, including those that are CD25+ CD4+ Foxp3+, as well as newly developed methods for inducing Treg expansion in vitro and in vivo, has excited the transplant community [58, 59]. While only limited success has thus far been achieved toward developing human allograft tolerance in humans, multiple groups are studying whether and how Tregs can be exploited to prolong graft survival and potentially induce robust allograft tolerance.

2.7 Summary

The field of solid organ transplantation has advanced considerably in the past half century, based largely on improved understanding of the mechanisms of allograft rejection and the parallel development of effective immunosuppressive drugs. Currently available immunosuppressive drugs are not completely effective in preventing or treating allograft rejection. Moreover, long-term treatment with these agents is associated with toxicities including infection and malignancy—topics that will be covered in detail elsewhere in this book. Thus, organ transplantation remains an imperfect modality. Effective strategies for creating true immune tolerance might allow organ transplantation without the use of immunosuppressive drugs. However, a breakthrough of that kind would only partially offset the most important limitation in the field: a continued shortage of organ donors.

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3 Immunosuppressive Agents

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3.1 Introduction

Over the last half century, the field of kidney transplant has experienced a significant change. From better insights into immunology, immunodiagnostics, and immunotherapeutics to refinements in the surgical processes, the course and expected outcomes for these patients have improved dramatically. The philosophy of therapeutic immunosuppression and the tools available to deliver it are steadily improving as well.

The basic profile of current immunosuppression strategy has remained fundamentally unchanged—two distinct phases can be teased out. "Induction" is the initial phase of heavy immunosuppression to avoid early acute rejection and allow for maintenance immunosuppression to become effective. The subsequent "maintenance" phase attempts at keeping the patient optimally immunosuppressed but at the same time limiting the fallout (infections, metabolic complications, malignancies, etc.) of the immunocompromised state. These two phases are periodically punctuated by a third "rescue" phase that is deployed should the patient suffer a rejection episode.

The alloimmune response is complicated and multipronged with several loops of redundancy. Over the years, many different drugs have been used in combination targeting various steps and pieces of the immune response. This sequentially arrests the immune response and has the added advantage of using lower doses of each individual drug allowing for an agreeable decrease in individual toxicities. This has led to the paradigm of combination drug regimens for the management of the kidney transplant recipient.

Despite significant gains in this respect, many problems remain. There are few reliable methods of measuring alloreactivity directly in widespread clinical use. This constrains transplant physicians to depend solely on indirect data (like blood counts and drug levels) and monitoring toxicities of the individual drugs being administered. Over-immunosuppression of a particular patient, drug-related toxicities, and side effects as well as opportunistic infections remain a daily hazard to the well-being of the transplant recipient. Indeed, increased cardiovascular mortality is one of the biggest challenges in managing the transplant patient.

3.2 Structure of Immunosuppressive Regimens

As noted, the immune response is complex and multipronged, replete with redundant loops. The purpose of immunosuppressive therapy remains avoidance of alloreactive responses and rejection of the allograft. Use of one agent alone may not protect against this alloreactivity due to the nature of the immune response or may be attended with unacceptable toxicity.

Combining drugs has been an effective strategy at targeting these complex processes at multiple points, creating synergy to achieve therapeutic effectiveness. A desired consequence is the ability to reduce exposure to each constituent drug in the regimen, decreasing individual drug toxicity as well as perhaps the overall degree of immunosuppression.

In the early days of transplantation, steroids and antiproliferative agents (azathioprine) formed the backbone of transplant immunosuppression regimens (Table 3-1). Introduction of calcineurin inhibitors (cyclosporine) revolutionized transplantation. The rates of acute rejection fell dramatically and the era of standard triple immunosuppression was ushered in—a calcineurin inhibitor anchor (cyclosporine and later tacrolimus), an adjuvant antimetabolite antiproliferative agent (azathioprine and later mycophenolic acid preparations), and steroids. This structure remains the most commonly used strategy for therapeutic immunosuppression today [1].

Identifying the critical nature of the first few months after transplantation from a rejection point of view, many centers add a layer of induction to the standard maintenance immunosuppression, especially to those thought to

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Table 3-1. Classification of immunosuppressive agents

Classification	Drug (generic)	Drug (trade)	Generic	Dosage form
IL-2 receptor blockers	Basiliximab	Simulect®	No	Injection
Anti-T-cell therapy	Antithymocyte globulin—horse	Atgam®	No	Injection
	Antithymocyte globulin—rabbit	Thymoglobulin®	No	Injection
CD52 antibody	Alemtuzumab	Campath®	No	Injection
Corticosteroids	Methylprednisolone	Solu-Medrol®	Yes	Injection, oral
	Prednisone	Deltasone®	Yes	Oral
Calcineurin inhibitors	Cyclosporine nonmodified, CsA	Sandimmune®	Yes	Injection, oral
	Cyclosporine modified, microemulsion	Neoral®	Yes	Injection, oral
	Tacrolimus, FK	Prograf®	Yes	Oral
	Extended-release tacrolimus	Astagraf®	No	Oral
MTOR inhibitors	Sirolimus, rapamycin	Rapamune®	No	Oral
	Everolimus	Zortress®	No	Oral
Antiproliferative	Azathioprine, AZA	Imuran®	Yes	Injection, oral
	Mycophenolate mofetil, MMF	Cellcept®	Yes	Injection, oral
	Mycophenolate sodium, EC-MPS	Myfortic®	Yes	Oral
Co-stimulation blockade	Belatacept	Nulojix®	No	Injection

be at high immunological risk. This is referred to as "quadruple immunosuppression." Polyclonal antibodies such as antithymocyte globulins or monoclonal antibodies, such as basiliximab and alemtuzumab, may be given as induction immunosuppression.

By effectively using these medicines, the rates of acute rejection have steadily dropped over the decades. Having achieved this goal, transplant physicians are now trying to refine these strategies to reduce the morbidity of these drugs as well as that of the immunosuppressed state. Calcineurin inhibitors have reduced acute rejection rates and have improved short-term allograft survival, but clear evidence of benefit on long-term allograft survival is not yet conclusive. These agents themselves have nephrotoxic potential and expose the recipient to harmful metabolic complications like diabetes, hypertension, and dyslipidemias, contributing to high cardiovascular morbidity and mortality. Thus, the search for alternate substitute agents led to mTOR inhibitors as well as co-stimulation blocking agents like belatacept. Calcineurin-free immunosuppression is the subject of intense current research but is not a dominant clinical trend yet.

Similarly, buoyed by the effectiveness of other agents available, many centers remove the steroid part of the standard triple immunosuppression protocols. Steroids are either completely eliminated (steroid-free regimens) or are withdrawn after a discreet but early interval posttransplant (avoidance). Monotherapy for maintenance immunosuppression is being studied but is rarely used outside of clinical trial protocols [2, 3]. Intuitively, this strategy would be reserved for patients with transplanted organs that are perceived to have less immunogenic potential and considered to be at less immunological risk.

With the many choices of drugs available, their side effect profiles (Table 3-2) as well as the ever-expanding complexity of immune risks, a one-size-fits-all strategy for therapeutic immunosuppression is no longer adequate or appropriate. Though "center protocols" still exist and with good reason, the emphasis now must be on immunosuppression individualization. A plan for each patient must be created prior to transplantation based on immunological risk and patient profile and then constantly adjusted based on the posttransplant course (Table 3-3).

3.3 Induction Immunosuppression

The first few months after transplantation are a time of heightened risk for alloimmune events. Passenger donor antigen-presenting cells cause acute rejection via the direct antigen presentation pathway. This is also the time when the recipient immune system is surveying the allograft antigens for the first time. The risk of acute rejection recedes after the first few months perhaps as the passenger antigen-presenting cells are eliminated.

To counter this early risk of rejection, a strategy to aggressively target T-cell signaling to control T-cell-mediated alloimmune responses in the early posttransplant period has become popular. Termed induction immunosuppression, this represents a period of intense immunosuppression afforded right before and immediately after kidney transplantation. Induction immunosuppression not only reduces acute rejection, but many other corollary benefits are derived as well, such as minimizing the doses necessary for other immunosuppressive agents.

Induction immunosuppression is afforded by deploying antibody-based therapy and is used in addition to usual antirejection medicines. Over the decades, use of induction immunosuppression has increased from less than 30 % of transplants in the 1980s to over 80 % in 2010 [1]. Many different types and preparations of these antibodies are available.

TABLE 3-2. Immunosuppressive medication doses and side effects

Drug	Dose	Side effects
Induction		
Basiliximab	20 mg IV × 2 doses	Hypersensitivity reactions
Antithymocyte globulin		
Rabbit	1.5 mg/kg IV × 3–7 days	Rash, fever, thrombocytopenia, leukopenia
Horse	15 mg/kg IV × 3–7 days	Rash, fever, thrombocytopenia, leukopenia
Alemtuzumab	30 mg IV × 1 dose[a]	Fever, chills, lymphopenia, neutropenia, anemia, thrombocytopenia, infection
Maintenance		
Prednisone	Maintenance: 2.5–10 mg/day Rejection: 250–1000 mg/day × 3 days IV	Mood disturbances, psychosis, cataracts, hypertension, fluid retention, peptic ulcers, osteoporosis, muscle weakness, impaired wound healing, glucose intolerance, weight gain
Cyclosporine	4–5 mg/kg po twice daily	Neurotoxicity, gingival hyperplasia, hirsutism, hypertension, hyperlipidemia, glucose intolerance, nephrotoxicity, electrolyte disturbances
Tacrolimus	0.05–0.075 mg/kg po twice daily	Neurotoxicity, alopecia, hypertension, hyperlipidemia, glucose intolerance, nephrotoxicity, electrolyte disturbances
Extended-release tacrolimus	0.1 mg/kg po twice daily	Neurotoxicity, alopecia, hypertension, hyperlipidemia, glucose intolerance, nephrotoxicity, electrolyte disturbances
Sirolimus	2–10 mg/day po daily	Hypertriglyceridemia, anemia, thrombocytopenia, mouth sores, hypercholesterolemia, gastrointestinal disturbances, bone marrow suppression, poor wound healing, edema
Everolimus	0.75 mg po twice daily	Hypertriglyceridemia, anemia, thrombocytopenia, mouth sores, hypercholesterolemia, gastrointestinal disturbances, bone marrow suppression, poor wound healing, edema
Azathioprine	1–2.5 mg/kg/day po daily	Leukopenia, thrombocytopenia, gastrointestinal disturbances, pancreatitis, hepatotoxicity
Mycophenolate mofetil	500–1500 mg po twice daily	Leukopenia, thrombocytopenia, gastrointestinal disturbances
Mycophenolate sodium	360–1080 mg po twice daily	Leukopenia, thrombocytopenia, gastrointestinal disturbances
Belatacept	10 mg/kg administered, prior to implantation, on day 5, and at the end of weeks 2, 4, 8, and 12, then 5 mg/kg every 4 weeks (plus or minus 3 days)	Edema, hypertension, diarrhea, anemia, infection, cough
Rejection		
Methylprednisolone	Rejection: 250–1000 mg/day × 3 day IV	Mood disturbances, psychosis, cataracts, hypertension, fluid retention, peptic ulcers, osteoporosis, muscle weakness, impaired wound healing, glucose intolerance, weight gain
Antithymocyte globulin		
Rabbit	1.5 mg/kg IV × 7–14 days	Rash, fever, thrombocytopenia, leukopenia
Horse	15 mg/kg IV × 7–14 days	Rash, fever, thrombocytopenia, leukopenia
Rituximab	375 mg/m^2 IV dosed to response[a]	Fever, fatigue, lymphopenia, anemia, infusion-related reactions, infection
Eculizumab	600 mg IV weekly for 6 doses[a]	Hypertension, headache, anemia, infection
Bortezumib	1.3 mg/m^2 IV days 1, 4, 8, and 12[a]	Fatigue, fever, diarrhea, nausea, gastrointestinal side effects, thrombocytopenia, neutropenia, peripheral neuropathy

PO by mouth, *IV* intravenously.
[a]Not indicated for transplantation.

3.3.1 Nondepleting Antibodies

Basiliximab is an interleukin (IL)-2 receptor antagonist and is the only Food and Drug Administration (FDA)-approved induction agent in renal transplantation. It demonstrated statistically significant reduction in the incidence of acute rejection in three landmark clinical trials, two of which used a maintenance regimen of cyclosporine and corticosteroids without an antimetabolite [4–6]. Using a more contemporary regimen, a trial comparing basiliximab to placebo (using cyclosporine, corticosteroids, and mycophenolate mofetil for maintenance) demonstrated a trend toward reduced incidence of acute rejection in the treatment group (15.3 % vs. 26.6 %), although it did not reach statistical significance [7]. None of these trials demonstrated a significant difference in patient or allograft survival. It is dosed at 20 mg intravenously intraoperatively and 4 days after transplantation. It has few adverse reactions or drug interactions.

Table 3-3. Tailoring immunosuppressive regimens on adverse events

Condition	Immunosuppressive cause	Immunosuppressive change
New-onset diabetes after transplantation	Corticosteroid, tacrolimus, cyclosporine, mTORS	Avoidance, dose reduction
Dyslipidemia	Corticosteroids, cyclosporine Sirolimus, everolimus	Avoidance, dose reduction, tacrolimus
Hypertension	Corticosteroid, cyclosporine, tacrolimus, mTORS	Avoidance, dose reduction
Osteoporosis	Corticosteroids	Avoidance, dose reduction
Bone marrow suppression	Mycophenolic acid, azathioprine, sirolimus, everolimus, tacrolimus	Dose reduction
Delayed wound healing	Sirolimus, everolimus	Avoidance
Gastrointestinal side effects	Mycophenolate mofetil, tacrolimus, sirolimus	Enteric-coated mycophenolic sodium, dose reduction, azathioprine
Proteinuria	Sirolimus, everolimus	Avoidance
Nephrotoxicity	Cyclosporine, tacrolimus, sirolimus	Avoidance, dose reduction, belatacept

Adapted from KDIGO clinical practice guideline for the care of kidney transplant recipients. Am J Transplant. 2009 Nov;9 Suppl 3:S1–155.

3.3.2 Depleting Antibodies

The use of depleting antibodies causes a profound depletion of lymphocytes and leads to a significant reduction in acute rejection. Two depleting antibody preparations are currently available in the USA—the polyclonal antithymocyte globulin(r-ATG, Thymoglobulin® and horse-ATG, ATGAM®) and the monoclonal humanized alemtuzumab. Polyclonal antibodies have specificities for various lymphocyte surface antigens and cause a prolonged and durable depletion in lymphocytes. It may take several months for these lymphocytes to repopulate.

The optimal dose for r-ATG is unclear. Most centers use 1.5 mg/kg intravenously for 3–5 days [8–16]. However, lower doses of r-ATG (0.5, 1, and 2 mg/kg for 3 days after transplantation) have been associated with dose-dependent lymphocyte depletion (in intensity and duration) and prevention of acute rejection [17]. Conversely, nonhuman primate data suggests a more significant impact on T-cell depletion in lymph nodes (but not thymus) with higher doses [18]. Higher induction doses of 6–9 mg/kg have been used intraoperatively with subsequent sparing of maintenance immunosuppression with success [19, 20]. Rabbit antithymocyte globulin can be associated with an infusion cytokine release syndrome and premedication is suggested. Careful monitoring of blood counts is essential. Leukopenia and thrombocytopenia are commonly seen and may mandate dosage changes or interruption.

Alemtuzumab is a humanized monoclonal antibody targeting CD52 present on T lymphocytes, B lymphocytes, and monocytes. It causes profound and sustained lymphocyte depletion. Originally developed as one of the agents for conditioning in bone marrow transplantation, it was later utilized as treatment for chronic lymphocytic leukemia. In kidney transplantation, it was introduced as an agent to treat rejection in 1995 [21]. It was later tried as an induction agent followed by minimal immunosuppression in the hopes of setting up near tolerance. This strategy revealed significant rejection when alemtuzumab was used as monotherapy or with minimal maintenance immunosuppression with a single agent [22]. It became apparent that despite profound lymphocyte depletion, alemtuzumab was not effective against memory T cells in sanctuary sites [23]. It has since found increasing use in conjunction with follow-up triple immunosuppression or double therapy (minimizing or avoiding steroids). Most commonly, it is used as a single dose of 30 mg intraoperatively. It can be dosed subcutaneously, but this route is not FDA approved. A minority of centers prefer two doses, while others still use a weight-based regimen. Alemtuzumab may also be associated with an infusion syndrome and prophylaxis is recommended.

Considerable knowledge may be gained from induction trials. Rabbit antithymocyte globulin and basiliximab were compared in two multicenter induction trials in combination with cyclosporine, mycophenolate mofetil, and corticosteroids. The first trial included low-immunological-risk patients and revealed similar incidence of acute rejection with similar patient and allograft survival at 12 months post-transplantation [24]. The second trial included moderate- to high-risk patients and demonstrated an improved combined endpoint for the incidence of rejection, allograft loss, and patient death in the r-ATG arm [25, 26]. Most of the benefit in combined endpoints was attributed to the decreased incidence of acute rejection. Alemtuzumab has also shown promise in lowering acute rejection rates in low-risk patients when compared to basiliximab [27]. Among high-risk patients, alemtuzumab and r-ATG had similar efficacy. In another randomized trial, patients treated with alemtuzumab without corticosteroids and mycophenolate/tacrolimus suffered less rejection but more polyoma infections than patients treated with basiliximab, corticosteroids, and mycophenolate/tacrolimus [28]. However, in both of these trials, the reduction in acute rejection episodes did not translate to improved allograft survival or improved renal function. A recent meta-analysis supports the results concluding that alemtuzumab induction reduces the risk of rejection compared with basiliximab but not r-ATG and recommends picking induction agents based on safety and cost [29].

3.4 Summary

There is significant disagreement on the preferred induction agent and many centers adhere to their own preferences and practice. However, based on available data, some general principles may be teased out. The choice of induction immunosuppression eventually must be weighed against two major factors: the assessed immunological risk and philosophy of maintenance immunosuppression. According to the KDIGO Clinical Practice Guidelines, basiliximab may be preferred in low-risk patients, and a depleting antibody (r-ATG or alemtuzumab) may be preferred in high-risk transplant patients [30] (Table 3-4). Similarly, regimens using immunosuppression-minimizing strategies or steroid avoidance may need induction with a depleting agent. Elderly patients, those with HCV infection, and patients with other risk factors (e.g., a history of malignancy) may be considered for nondepleting antibody induction to mitigate infection or cancer risk. This remains a complicated clinical decision and must be individualized and given careful consideration.

3.5 Maintenance Immunosuppression

After a few weeks in the recipient environment, the passenger antigen-presenting cells are slowly eliminated, and the mechanism of alloimmunity switches to the more traditional indirect antigen presentation. Acute rejection may still occur but is not as common except as a consequence of noncompliance. The main concern at this stage is the development of chronic alloimmune rejection, a largely antibody-mediated rejection process. At this stage, the immune system needs to be under constant check. The emphasis remains on controlling CD4+ T-cell responses as well as immune cell recruitment and proliferation. As this is a long-term process, this has to be done suavely to avoid drug toxicity, opportunistic infection, and cancer.

TABLE 3-4. Summary of renal transplant clinical practice guidelines

Induction therapy
1. We recommend starting a combination of immunosuppressive medications before, or at the time of, kidney transplantation (1A)
2. We recommend including induction therapy with a biologic agent as part of the initial immunosuppressive regimen in KTRs (1A)
3. We recommend that an IL2-RA be the first-line induction therapy (1B)
4. We suggest using a lymphocyte-depleting agent, rather than an IL2-RA, for KTRs at high immunological risk (2B)
Initial maintenance immunosuppressive medications
1. We recommend using a combination of immunosuppressive medications as maintenance therapy including a CNI and an antiproliferative agent, with or without corticosteroids (1B)
2. We suggest that tacrolimus be the first-line CNI used (2A)
3. We suggest that tacrolimus or CsA be started before or at the time of transplantation, rather than delayed until the onset of allograft function (2D tacrolimus; 2B CsA)
4. We suggest that mycophenolate be the first-line antiproliferative agent (2B)
5. We suggest that, in patients who are at low immunological risk and who receive induction therapy, corticosteroids could be discontinued during the first week after transplantation (2B)
6. We recommend that if mTORi are used, they should not be started until allograft function is established and surgical wounds are healed (1B)
Long-term maintenance immunosuppressive medications
1. We suggest using the lowest planned doses of maintenance immunosuppressive medications by 2–4 months after transplantation, if there has been no acute rejection (2C)
2. We suggest that CNIs be continued rather than withdrawn (2B)
3. If prednisone is being used beyond the first week after transplantation, we suggest prednisone be continued rather than withdrawn (2C)
Treatment of acute rejection
1. We recommend corticosteroids for the initial treatment of acute cellular rejection (1D)
2. We suggest adding or restoring maintenance prednisone in patients not on steroids who have a rejection episode (2D)
3. We suggest using lymphocyte-depleting antibodies for acute cellular rejections that do not respond to corticosteroids and for recurrent acute cellular rejections (2C)
4. We suggest treating antibody-mediated acute rejection with one or more of the following alternatives, with or without corticosteroids (2C): plasma exchange, intravenous immunoglobulin, anti-CD20 antibody, lymphocyte-depleting antibody
5. For patients who have a rejection episode, we suggest adding mycophenolate if the patient is not receiving mycophenolate or azathioprine or switching azathioprine to mycophenolate (2D)

IL2-RA interleukin 2 receptor antagonist, *KTRs* kidney transplant recipients, *CNI* calcineurin inhibitor, *CsA* cyclosporine A, *mTORi* mammalian target of rapamycin inhibitor(s).

Adapted from KDIGO clinical practice guideline for the care of kidney transplant recipients. Am J Transplant. 2009 Nov;9 Suppl 3:S1–155.

As noted above, most centers choose to use a calcineurin inhibitor anchor (either tacrolimus or cyclosporine) along with an adjuvant agent (azathioprine or a mycophenolic acid preparation) with or without steroids. Other agents in use are mTOR inhibitors and co-stimulation blocking agents (belatacept).

3.5.1 Calcineurin Inhibitors

Calcineurin inhibitors are the first act in the modern transplantation miracle. They directly target the lymphocytes nonlethally by restricting the ability of CD4 T cells to produce IL-2, the vaunted signal three of T-cell activation limiting immune cell proliferation in response to activation of CD4 cells. This revolutionized kidney and other organ transplantation. Acute rejection rates plummeted markedly improving short-term patient and allograft survival.

Cyclosporine was the first agent in this class to be approved by the FDA in 1983. The earliest formulation of cyclosporine, cyclosporine nonmodified, is as an aquaphobic, softgel capsule. Subsequently, a microemulsion form (cyclosporine modified) was introduced. In the late 1980s, the second agent tacrolimus surfaced. It was approved by the FDA in 1994 for use in liver transplantation and has been used in all forms of transplantation since. A prolonged-release form of tacrolimus has now been introduced into clinical practice.

Early studies that compared microemulsion cyclosporine (modified) to tacrolimus using the combination of calcineurin inhibitors, azathioprine, and corticosteroids demonstrated a significant decrease in acute rejection with tacrolimus, while there was no difference in patient or allograft survival posttransplantation [31, 32]. A more recent study randomized first deceased donor recipients to one of three immunosuppressive regimens (all included corticosteroids): (1) tacrolimus with azathioprine, (2) tacrolimus with mycophenolate mofetil, and (3) microemulsion cyclosporine and mycophenolate mofetil [33]. Acute rejection rates were similar in each group, but the incidence of corticosteroid resistant rejection was lower in the tacrolimus arms. At 3 years overall renal function, patient survival, and allograft survival remained the same, but in the tacrolimus arms, improved allograft survival was seen in recipients with delayed allograft function [33]. In agreement with this data, a meta-analysis reported that for every 100 patients treated with tacrolimus rather than cyclosporine for the first year, 12 would be prevented from having acute rejection, two would be prevented from having allograft failure, but five would develop new-onset diabetes after transplantation [34]. Furthermore, the Elite-Symphony trial demonstrated that a low-dose cyclosporine regimen was not as effective as a low-dose tacrolimus regimen [35]. As a result of these trials, the KDIGO Clinical Practice Guidelines suggest that tacrolimus should be the first-line calcineurin inhibitor for renal transplant recipients [30]. Clinical trends have followed the science. Over the past two decades, tacrolimus has become overwhelmingly the more popular calcineurin inhibitor in use for kidney transplant patients

Both calcineurin inhibitors can be given intravenously or orally. They have a narrow therapeutic index and need to be monitored closely. For both cyclosporine and tacrolimus, 12-h trough levels are commonly followed. Target 12-h trough levels of 100–250 ng/mL for cyclosporine and 5–15 ng/mL for tacrolimus are typical soon after transplantation. Subsequently levels are reduced. Two-hour peak levels (C2 levels) are followed by some centers for cyclosporine but not for tacrolimus. The reference levels for these drugs given are generalizations and vary significantly between patients based on the anticipated immune risk, structure of the immunosuppression regimen (especially induction), and recipient factors like age, infection threat, and history or risk of malignancy. Calcineurin inhibitors are metabolized via the CYP3A4 pathway, predisposing them to many potentially serious drug interactions.

Despite careful therapeutic drug monitoring, many side effects may be encountered. Hirsutism, gingival hypertrophy, hypertension, and hyperlipidemia are more commonly seen with cyclosporine, whereas neurotoxicity, alopecia, and posttransplant diabetes are more commonly associated with tacrolimus.

Adherence is essential to prevent poor outcomes after transplantation. For this reason, a prolonged-release tacrolimus formulation was developed. Two pivotal trials that compared prolonged-release tacrolimus to immediate-release tacrolimus have shown comparable efficacy and safety [36, 37]. Various studies have suggested that the tacrolimus levels are slightly lower (10–20 %) with prolonged-release tacrolimus group compared to twice daily tacrolimus patients [38, 39]. There may be an increased risk of acute rejection with prolonged-release tacrolimus [36, 37] and close monitoring is warranted. A new formulation of prolonged-release tacrolimus, LCP-tacro, may become commercially available in 2016.

3.5.2 Corticosteroids

Corticosteroids were one of the earliest immunosuppressive medicines to be widely used. Almost simultaneously, the adverse effects of this use became apparent. Steroids have been implicated in increased rates of new-onset diabetes after transplantation, hypertension, and dyslipidemia. This in turn is thought to contribute to the very high cardio- and cerebrovascular mortality and morbidity in kidney transplant patients. Additionally, steroid use is thought to contribute to osteopenia and metabolic bone disease seen in these patients.

Despite this negative side effect profile, corticosteroids have proven themselves to be effective immunosuppressive agents. This is due to the myriad and multilevel effects they exert on the immune response, unlike most other agents that

target one specific pathway. Steroids, on internalization, bind to receptors and affect transcription of various genes, collectively referred to as the glucocorticoid responsive element (GRE). This genomic trans-suppression is responsible for most of the salutary anti-inflammatory effects of steroids [40]. Additional non-genomic mechanisms also contribute to reduced T-cell activation [41]. This leads to proliferation restriction and signaling changes that affect both T cells and B cells. Additionally, they impact lymphocyte trafficking by sequestering CD4 cells in the lymphatic organs.

This multilevel and multifaceted impact of steroids on the immune response made early efforts to limit or replace them tricky. In the first double-blinded, randomized, placebo-controlled, multicenter, 5-year trial comparing early steroid discontinuation (7 days posttransplant) with steroid maintenance therapy, the rate of biopsy-proven acute rejection and chronic allograft nephropathy was twice as high in the rapid corticosteroid discontinuation arm [42]. In early studies, salutary metabolic effects were deemed marginal, but a subsequent meta-analysis confirmed significant reduction in these cardiovascular risk factors in patients whom corticosteroids are avoided or withdrawn [43]. Three pooled studies of corticosteroid withdrawal have shown that despite the increased incidence of acute rejection in the withdrawal arms, short-term results demonstrate comparable patient and allograft survival [44–46].

The best candidates for steroid withdrawal are low immunologic patients conditioned with induction therapy, especially those at risk for corticosteroid adverse effects [30]. In studies of steroid withdrawal, African Americans have a much higher incidence of acute rejection [47, 48] and together with other high-immunological-risk subgroups may not be the best candidates for such withdrawal.

Timing of steroid withdrawal is critical. If withdrawal is contemplated, it should be completed in the first week after transplantation. If prednisone is being used beyond the first week after renal transplantation, it should be continued rather than withdrawn.

3.5.3 Antimetabolite Agents

Antimetabolite agents include azathioprine and mycophenolic acid. Azathioprine is a purine analog that inhibits DNA replication and suppresses B- and T-cell proliferation. Typical doses of azathioprine range from 1 to 2.5 mg/kg/day. Adverse effects of azathioprine are dose-related bone marrow suppression and gastrointestinal disturbances. Other rare, but serious, adverse events, like pancreatitis and elevations in liver function tests, paired with a potential serious drug interaction with allopurinol have limited the use of azathioprine. Mycophenolic acid causes noncompetitive reversible inhibition of inosine monophosphate dehydrogenases (IMPDH). This interferes with the de novo pathway of purine synthesis and DNA replication, producing cytostatic effects on T and B cells. Mycophenolic acid is available as mycophenolate mofetil or enteric-coated mycophenolic sodium. Mycophenolate mofetil is rapidly converted to mycophenolic acid in the liver, and enterohepatic recirculation of mycophenolic acid may occur. Typical doses of mycophenolate mofetil range from 500 to 1500 mg orally twice daily. A dose of 250 mg of mycophenolate mofetil is equivalent to 180 mg of enteric-coated mycophenolic sodium. Magnesium and zinc containing products should not be co-administered with mycophenolic acid. Common adverse effects of mycophenolate mofetil include dose-related gastrointestinal side effects, leukopenia, and thrombocytopenia.

The efficacy of mycophenolate mofetil in renal transplantation has been reported in several well-designed trials [49–53]. Mycophenolate mofetil treatment groups demonstrated a reduced incidence and severity of early rejection episodes as compared to low-dose azathioprine-treated patients with treatment regimens consisting of tacrolimus plus corticosteroid as well as cyclosporine plus corticosteroid [49]. Three-year follow-up of these studies found that the decreased incidence of early rejection in the mycophenolate mofetil arm had not translated into a significant improvement in allograft function or survival [50, 51]. As a result of the summative evidence from these trials, the KDIGO Clinical Practice Guidelines suggest that mycophenolate be the first-line antiproliferative agent in kidney transplant recipients [30].

Enteric-coated mycophenolate sodium was developed with the hopes of avoiding the upper gastrointestinal side effects of mycophenolic acid by facilitating drug release in the small intestine [54]. Two major clinical trials demonstrated that enteric-coated mycophenolate sodium is therapeutically equivalent to mycophenolic mofetil, yet they did not demonstrate a statistically significant difference in overall gastrointestinal symptoms [55, 56]. Many post-marketing studies have proven a beneficial effect of enteric-coated mycophenolate sodium, while others have not reported a difference in gastrointestinal-related adverse effects [57–69]. Gastrointestinal events are multifactorial and may be related to multiple factors including infectious etiology, gastroparesis, or concomitant medications. Enteric-coated mycophenolate sodium may offer benefit to specific populations. However, enteric coating is unlikely to influence the systemic effects of the drug including effects on enterocyte proliferation and viral infection that may be responsible for gastrointestinal side effects.

3.5.4 mTOR Inhibitors

An alternative to the calcineurin inhibitor-based regimens is a mTOR inhibitor-based regimen. Sirolimus and everolimus bind to FKBP-12 to form an immunosuppressive complex which inhibits the regulatory kinase, mTOR. This inhibition suppresses cytokine-mediated T-cell proliferation, halting

progression from the G1 to the S phase of the cell cycle. Sirolimus is dosed orally once daily (typically 2–5 mg/day) with adjustments based on target trough levels of 5–15 ng/mL. Everolimus is a sirolimus derivative with a much shorter half-life that is approved for the treatment of advanced renal cell carcinoma and renal and liver transplantation. Everolimus, initially dosed at 0.75 mg orally twice daily followed by adjustment to target serum drug concentration between 3 and 8 ng/mL, has an adverse event profile similar to sirolimus. Sirolimus is a substrate of CYP3A4 and everolimus is an inhibitor of CYP3A4. In kidney transplantation, the mTOR inhibitors, sirolimus and everolimus, have been associated with delayed allograft function; thus, the KDIGO Clinical Practice Guidelines recommend that if a mTOR inhibitor is used, it should not be started until allograft function is established and surgical wounds are healed [30]. Although sirolimus has a relatively low risk of traditional afferent arteriolar vasoconstrictive nephrotoxicity, concomitant use of angiotensin-converting enzyme inhibitors (ACEI) may cause acute renal failure [70] or anaphylaxis [71]. Sirolimus is uncommonly used early after lung transplant due to associated risk of anastomotic bronchial dehiscence [72, 73]. It has also been associated with hepatic artery thrombosis following liver transplant [74].

The de novo use of sirolimus has been proven to be comparable to a calcineurin inhibitor as seen in the ORION trial and the Spare-the-Nephron trial, while it has been associated with early posttransplant adverse events including lymphoceles, prolonged delayed allograft function, and poor wound healing [75–78]. Likewise de novo use of everolimus in combination with induction has produced acceptable rates of acute rejection, although adverse events were common [79, 80]. Based on data from the CONVERT trial, it appears that sirolimus conversion is only successful in a subgroup of patients with a baseline glomerular filtration rate more than 40 mL/min and urine protein to urine creatinine ratio less than or equal to 0.11 [81]. Likewise, the ZEUS study demonstrated the everolimus conversion is possible in low- to moderate-risk patients with normal renal function, although this may come at the expense of a higher acute rejection rate. The best evidence for calcineurin withdrawal with mTOR inhibitors is in selected patients. Close monitoring of drug concentration levels and adverse events is warranted. Whether or not calcineurin inhibitor-free/sparing regimens using mTOR inhibitor maintenance therapy is efficacious in the long term remains unknown. Therefore at this time, the KDIGO Clinical Practice Guidelines suggest that calcineurin inhibitors be continued rather than withdrawn [30].

3.5.5 Belatacept

Belatacept is a second-generation co-stimulation blocker that is administered as a well-tolerated intravenous infusion over 30 min. The recommended dosing is 10 mg/kg administered, prior to transplantation, on day 5 and at the end of weeks 2, 4, 8, and 12, then 5 mg/kg every 4 weeks (plus or minus 3 days).

Belatacept has been studied in several clinical renal transplantation trials. In a phase 2 study, more-intensive belatacept, less-intensive belatacept, and cyclosporine produced similar rates of acute rejection and allograft loss, while the glomerular filtration was statistically higher in each of the belatacept arms [82, 83]. Two phase three trials of de novo kidney transplant recipients tested the efficacy and safety of belatacept [84–86]. In both trials, patients were randomized into three groups: more-intensive belatacept, less-intensive belatacept, and cyclosporine in conjunction with basiliximab, mycophenolate, and corticosteroids. BENEFIT-EXT was designed similarly to the BENEFIT trial with the inclusion of expanded criteria donors. In the BENEFIT trial, despite the higher incidence of acute rejection in the belatacept arm, renal function was superior in the belatacept arms. In the BENEFIT-EXT trial, acute rejection rates were similar and renal function was statistically superior in the more-intensive belatacept group, but not the less-intensive group [85]. Three-year follow-up of these trials demonstrated persistent improvement in renal function (mean change +21 mL/min BENEFIT and +10 mL/min BENEFIT-EXT) [86]. Unfortunately, there was a high incidence of posttransplant lymphoproliferative disease in the belatacept-treated, Epstein–Barr virus seronegative recipient arms, and therefore, the drug is contraindicated in patients that are Epstein–Barr virus seronegative. One limitation of the BENEFIT and BENEFIT-EXT trials was that in the comparison arm, cyclosporine, a less contemporary immunosuppressant, was used at high doses and concentrations. Addressing this issue, a smaller trial used a more contemporary immunosuppressive regimen of belatacept/mycophenolate mofetil, belatacept/sirolimus, and tacrolimus/mycophenolate mofetil, in combination with r-ATG without corticosteroids [87]. Acute rejection was highest in the belatacept/mycophenolate mofetil arm, allograft loss was lowest in the tacrolimus/mycophenolate arm, and renal function was better in the belatacept arms.

A randomized, conversion trial has tested the hypothesis that belatacept-based regimens may provide a treatment option for calcineurin-based maintenance immunosuppression [88]. The relative renal benefit of belatacept was observed in patients switched from either cyclosporine (+7.7 mL/min) or tacrolimus (+6.4 mL/min). Patient survival, allograft survival, and the overall safety profile were similar between groups. Close monitoring of patients is necessary as six patients in the belatacept group had acute rejection episodes, all of them within the first six months after conversion.

Belatacept is the first immunosuppressive agent to demonstrate an improvement in glomerular filtration rate over a calcineurin inhibitor-based regimen. The chronic intravenous administration and drug cost may influence prescribing patterns and patient compliance. Further trials are needed to

explore the long-term outcomes. These trials should include more current immunosuppressive regimens.

3.5.6 Summary

In the past two decades, tacrolimus and mycophenolic acid have become the cornerstones of immunosuppressive regimens. The KDIGO Clinical Practice Guidelines recommend using a combination of immunosuppressive medications as maintenance therapy including a calcineurin inhibitor and an antiproliferative agent, with or without corticosteroids [30]. They suggest that tacrolimus be the first-line calcineurin inhibitor used and that mycophenolate be the first-line antiproliferative agent. In patients who are at low immunological risk and who receive induction therapy, corticosteroids could be discontinued during the first week after transplantation.

3.6 Treatment of Rejection

In the current era of transplantation, balancing the need for immunosuppression to prevent allograft rejection while minimizing drug toxicity, infections, and malignancy continues to be a challenging task. Potent immunosuppressive agents have significantly lowered the incidence of acute cellular rejection in the first years after transplantation. Antibody-mediated rejection continues to threaten the long-term survival of the allografts.

3.6.1 Acute Cellular Rejection

Corticosteroids are generally considered first line for treatment of cellular rejection. Methylprednisolone 0.5–1.0 g intravenously is given once daily for 3 days [89, 90], then oral corticosteroids are tapered rapidly to the pretreatment dose. If a patient was steroid-free prior to the rejection episode, many centers will continue the corticosteroids for the life of the allograft. Anti-T-cell antibodies, which remove functional T cells from the circulation, can be used when corticosteroids have failed to reverse rejection, for moderate to severe rejection, or for treatment of a recurrent rejection. Currently, there are two available antithymocyte globulins: one derived from horses and the other from rabbits. In a multicenter, double-blind, randomized trial, r-ATG demonstrated improved efficacy when compared to equine-derived antithymocyte globulin in the treatment of rejection [91]. Rabbit antithymocyte globulin, the most commonly used preparation, is dosed 1.5 mg/kg for 7–14 days and administered as an intravenous infusion delivered over a 4–6-h period preferably through a proximal central venous catheter, a peripherally inserted central catheter (PICC) line, or a high-flow vein with a 0.22 micron filter. Premedication including corticosteroids, acetaminophen, and an antihistamine is recommended to avoid infusion-related side effects and cytokine release syndrome. Other common adverse effects are leukopenia and thrombocytopenia which can be managed through dose adjustments.

Adjusting a patient's maintenance regimen may also aid in treating an acute rejection episode. If the drug levels are subtherapeutic prior to diagnosis, then the calcineurin inhibitor dose should be adjusted to reach the target range. Switching from cyclosporine to tacrolimus may also be beneficial [92]. A change to the patient's antimetabolite should also be considered. Increasing the antimetabolite dose is recommended if the patient can tolerate the higher-dose regimen.

3.6.2 Antibody-Mediated Rejection

Historically, antibody-mediated rejection has been very difficult to reverse and has not been well studied. Acute antibody-mediated rejection is less responsive to conventional antirejection therapy and has a worse prognosis than acute cellular rejection. Treatment regimens may include one or more of the following: antilymphocyte therapy, plasmapheresis, intravenous immunoglobulin (IVIG), and rituximab [93–99]. One cohort study comparing these strategies (plasmapheresis/IVIG/rituximab vs. IVIG alone) demonstrated improved allograft survival in the combination group [99]. The KDIGO Clinical Practice Guidelines suggest treating antibody-mediated acute rejection with one or more of the following alternatives with or without corticosteroids: plasma exchange, IVIG, anti-CD20 antibody, and lymphocyte-depleting antibody (grade 2C recommendation) [30]. Bortezomib and eculizumab may play a major role in antibody-mediated therapy, but more clinical data and well-designed clinical trials are needed.

3.6.3 Summary

Treatment for mild cellular rejection involves corticosteroids, whereas moderate to severe cellular rejection is typically treated with antithymocyte globulins. Humoral rejection is more difficult to treat and typically is treated with multiple therapies including IVIG, plasmapheresis, corticosteroids, and rituximab, eculizumab, or bortezomib.

3.7 Immunosuppression in Other Solid Organs

3.7.1 Pancreas Transplantation

In clinical practice, a maintenance immunosuppressive regimen containing a calcineurin inhibitor, mycophenolic acid, and corticosteroids is the preferred regimen for pancreas transplant recipients [100–102]. There is less evidence supporting the use of corticosteroid withdrawal in pancreas transplantation [103–112]. According to Scientific Registry

of Transplant Recipients (SRTR) data, 38 % of pancreas transplant recipients undergo corticosteroid withdrawal [1]. Little is known about the use of mTOR inhibitors in pancreas transplant recipients. Sirolimus appears to prevent rejection in pancreas transplant recipients, but only a few small, nonrandomized studies have been published [44, 45, 113–118].

3.7.2 Lung Transplantation

For the past two to three decades, the most commonly used regimen in lung transplant recipients has consisted of a calcineurin inhibitor plus an antimetabolite and corticosteroids [1]. There is less evidence supporting the use of tacrolimus in lung transplantation [119–122], and therefore the switch from cyclosporine to tacrolimus has been delayed when compared to other transplanted organs. Similarly, many lung transplant centers have not switched from azathioprine to mycophenolic acid [123–127]. Currently based on SRTR statistics, 86 % of new lung transplants are prescribed tacrolimus and 55 % of lung transplant recipients are given mycophenolic acid [1], perhaps because the published literature suggests an increased incidence of infection and gastrointestinal toxicity with mycophenolate mofetil [127]. Sirolimus is not commonly used or FDA approved for use in lung transplantation because it has been associated with several fatal cases of anastomotic bronchial dehiscence when used in the early posttransplantation period as well as interstitial pneumonitis. In contrast, everolimus has been studied in lung transplantation with more success [128, 129].

3.7.3 Liver Transplantation

For liver transplant recipients, the regimen that is most commonly used at the time of transplant is tacrolimus plus mycophenolic acid and corticosteroids, although corticosteroid withdrawal is becoming more common [1]. Many patients receive triple immunosuppressive therapy at the time of transplant, but agents are commonly weaned and dual or single therapy is commonplace. Lower acute rejection rates have been seen with tacrolimus (vs. cyclosporine) [130–132] and mycophenolate mofetil [133–135]. Corticosteroid withdrawal or avoidance has been attempted in liver transplant recipients in an effort to reduce adverse events and minimize the consequences of hepatitis C recurrence [136, 137]. Meta-analyses have concluded that when corticosteroids were replaced by other agents, the incidence of acute rejection was reduced and that corticosteroid-free regimens are beneficial in lowering cholesterol, cytomegalovirus infection, hypertension, and new-onset diabetes mellitus [138, 139]. Sirolimus is not approved in liver transplantation due to a higher rate of hepatic artery thrombosis when compared to cyclosporine. The use of sirolimus after 1 year after transplant as conversion therapy may be safe, although not effective as in preventing nephrotoxicity [140]. Everolimus may show more promise in patients converted for renal dysfunction, but adverse events may limit it use [141].

Heart Transplantation

Tacrolimus is the most widely used calcineurin inhibitor (75 %) in heart transplantation, mycophenolic is the predominant antimetabolite agent (88 %), and the use of sirolimus and everolimus remains low. Most patients (89 %) remain on low-dose glucocorticoids at 1-year posttransplantation. Single-center and multicenter randomized comparisons between de novo use of tacrolimus and cyclosporine after heart transplantation support the use of tacrolimus [142, 143]. The substitution of mycophenolate mofetil for azathioprine may reduce mortality and rejection in the first year after cardiac transplantation [144]. Enteric-coated mycophenolic sodium and mycophenolate mofetil appear to be similar except that significantly fewer enteric-coated mycophenolic sodium patients required dose reductions during treatment [145]. The mTOR inhibitors may be used in patients with cardiac allograft vasculopathy or renal insufficiency because of their inhibitory effects on smooth muscle proliferation and absence of intrinsic nephrotoxicity [146]. The high incidence of adverse effects, including lower-extremity edema and poor wound healing, may limit the universal use of these agents. Of the two available mTOR inhibitors, everolimus has better evidence of reducing acute rejection rates and preventing vasculopathy [147–149]. Corticosteroids are used in most heart transplant recipients at relatively high doses in the early postoperative period then tapered to low doses or discontinued altogether in the first transplant year [150, 151]. Low-risk patients may tolerate earlier (within 1–2 months posttransplantation) corticosteroid withdrawal without long-term adverse consequences [152, 153], but this may result in higher acute rejection rates and additional steroids during follow-up [154].

3.7.4 Intestinal Transplantation

Intestinal transplant recipients have the highest rejection rates and the lowest allograft survival rates due to the high immunogenicity of the bowel. Newer immunosuppressive drugs have played a significant role in the success with the procedure since the mid-1990s. Currently, most intestinal transplant recipients receive tacrolimus and corticosteroids as maintenance immunosuppression. Fewer than

200 intestine transplants are performed yearly and therefore the clinical trials of immunosuppression are few in number [155].

3.8 Conclusion

Induction, maintenance, and treatment of rejection are the three phases of immunosuppression. The choice of induction agent remains debatable and should be based on patient-specific risk factors. Basiliximab or no induction agent may be preferred in low-risk patients and r-ATG may be preferred in high-risk transplant patients. Maintenance therapy typically includes a calcineurin inhibitor and an antiproliferative agent, with or without corticosteroids. Tacrolimus and mycophenolate are the preferred maintenance agents. In patients who are at low immunological risk, corticosteroids can be avoided or withdrawn, although this practice is debatable. Treatment for mild cellular rejection involves corticosteroids, whereas moderate to severe cellular rejection is typically treated with antithymocyte globulins. Humoral rejection is treated with multiple therapies including IVIG, plasmapheresis, corticosteroids, rituximab, eculizumab, or bortezomib.

While awaiting further advances in the immunosuppressive armamentarium, we should be able to improve patient and allograft survival by tailoring available immunosuppressive agents. Maintaining the effectiveness of immunosuppressive therapy requires shifting our therapeutic approach from “one-size-fits-all” to a tailored or individualized strategy. In the current era of transplantation, immunosuppressive regimens are selected based on several factors including immunological risk of rejection, potential for excessive immunosuppression (e.g., infection and cancer), medication side effects, patient adherence, patient characteristics, cost, and presence of comorbid disease states. Balancing the need for immunosuppression to prevent allograft rejection while minimizing drug toxicity and the risk of infections and malignancy continues to be a challenging task. Given toxicities of immunosuppressive agents, different maintenance regimens are being explored to minimize adverse short- and long-term effects. Patients at risk for rejection may require more potent immunosuppressive medication, more medications, and higher doses. Patient at lower risk for rejection may need less potent medications, lower drug concentrations and dosages, or mono- or dual therapy.

Ongoing vigilant monitoring of transplant patients should be combined with a willingness to respond to rejection or infection with alterations in maintenance immunosuppressive therapy. Further study of these immunosuppressive regimens and novel agents should provide innovative strategies that extend the functional life of allografts and provide the ideal immunosuppressive regimen.

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4
Common Drug Interactions Encountered in Treating Transplant-Related Infection

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The treatment of patients with transplant-related infections requires close attention to the host in order to ensure adequate and safe dosing of anti-infective medications. Solid organ transplant recipients are at risk for synergistic toxicities due to drug–drug interactions between anti-infective agents and immunosuppressive medications. These toxicities relate to consequences of high exposures to the immunosuppressive and/or the anti-infective medication. While perhaps less frequent, the risk of inadequate anti-infective drug exposure and resulting treatment failure due to drug–drug interactions must also be avoided. This chapter presents a summary of the clinically significant drug–drug interactions encountered in providing anti-infective chemotherapy to solid organ transplant recipients.

Medications used for immunosuppression after organ transplantation can be split into seven categories (Table 4-1): polyclonal antibodies, monoclonal antibodies, calcineurin inhibitors, antimetabolites, mammalian target of rapamycin (mTOR) inhibitors, corticosteroids, and selective T-cell costimulation blockers. The reader is referred to Chap. 3 for more detailed information regarding the mechanism of action of these commonly used immunosuppressive agents. The risk of drug interactions is high, particularly within the calcineurin inhibitor and mTOR inhibitor drug classes.

Cyclosporine (Neoral, Sandimmune, Novartis Pharmaceuticals) and tacrolimus (Prograf, Astagraf XL, Astellas Pharmaceuticals) are calcineurin inhibitors. They suppress the immune system by blocking IL-2 signaling between immune cells. Major toxicities include electrolyte disturbances (i.e., hypophosphatemia, hypomagnesemia, hyperkalemia), hypertension, hyperlipidemia, hyperglycemia, nephrotoxicity, neurotoxicity, and others [1, 2]. Doses are adjusted to obtain target whole blood cyclosporine or tacrolimus levels and the target range is patient-specific.

Sirolimus (Rapamune, Pfizer Inc.) and everolimus (Zortress, Novartis Pharmaceuticals) are mTOR inhibitors whose actions inhibit T-cell activation and proliferation. Major toxicities include impaired wound healing, hypertriglyceridemia, hyperlipidemia, oral ulcers, proteinuria, and noninfectious pneumonitis [3–6]. Sirolimus and everolimus doses are adjusted to obtain target whole blood trough concentrations, and the target range is patient-specific. Sirolimus has a long half-life and it will take 1–2 weeks to reach steady state after initiating therapy and/or after dose changes.

Cyclosporine, tacrolimus, sirolimus, and everolimus are substrates for both cytochrome P-450 3A4 (CYP3A4) and P-glycoprotein (P-gp). Drugs and substances that induce CYP3A4 and/or P-gp may decrease cyclosporine/tacrolimus/sirolimus/everolimus concentrations, whereas inhibitors of CYP3A4 and/or P-gp may increase cyclosporine/tacrolimus/sirolimus/everolimus concentrations. Cyclosporine also inhibits CYP3A4 and P-gp and may have additional unique drug interactions not present with tacrolimus, sirolimus, and everolimus.

Azathioprine (Imuran; Prometheus Laboratories) is an antimetabolite. Major toxicities include bone marrow suppression manifesting as leukopenia, thrombocytopenia, and anemia. Standard, weight-based dosing is used for azathioprine; serum drug concentrations are not utilized [7].

Mycophenolate mofetil (MMF, CellCept; Genentech) and mycophenolic acid (MPA, Myfortic; Novartis Pharmaceuticals) are also antimetabolites, but, compared with azathioprine, their mechanism of action is more targeted to the white blood cell lines. Major toxicities include leukopenia and gastrointestinal adverse effects, most notably diarrhea [8]. Although these drugs are generally used at standard doses, therapeutic drug monitoring may be useful for MMF.

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MMF is a prodrug that, after absorption, is quickly hydrolyzed to MPA. Myfortic is an enteric coated formulation of MPA, which is the active moiety of both these drugs. The major metabolite of MPA, an MPA glucuronide known as MPAG, is excreted into urine and bile. Once in the GI tract, MPAG is converted back into MPA and reabsorbed, resulting in enterohepatic recirculation.

MMF and MPA are believed to have similar drug interactions. Indeed, most MPA drug interactions are derived from the MMF literature. The clinical impact may not be exactly the same, however, because each of these medications has a unique pharmacokinetic profile. In addition to the interactions listed in Table 4-2, drugs that alter the GI flora may disrupt enterohepatic recirculation of MPA. This is because natural GI flora is responsible for conversion of MPAG to MPA [75].

TABLE 4-1. Immunosuppressive agents [7]

Class	Generic name	Brand name
Calcineurin inhibitors	Cyclosporine	Neoral; Sandimmune
	Tacrolimus	Prograf; Astagraf XL
Antimetabolites	Azathioprine	Imuran
	Mycophenolate mofetil	CellCept
	Mycophenolic acid	Myfortic
Corticosteroids	Prednisone	Deltasone
mTOR inhibitors	Sirolimus	Rapamune
	Everolimus	Zortress
Polyclonal antibodies	Antithymocyte globulin, rabbit	Thymoglobulin
Monoclonal antibodies	Basiliximab (anti-CD25)	Simulect
	Alemtuzumab (anti-CD52)	Campath-1H
Selective T-cell costimulation blocker	Belatacept	Nulojix

For more information, also see Chap. 3.
Abbreviation: *mTOR* mammalian target of rapamycin.

There is scant evidence suggesting that corticosteroids may have clinically relevant drug interactions with other immunosuppressive agents [76]. Corticosteroids induce the CYP3A4 and P-gp pathways to varying degrees; cyclosporine, tacrolimus, sirolimus, and everolimus rely on these pathways for metabolism. Corticosteroids also induce uridine diphosphate glucuronosyltransferase enzymes and multidrug resistance-associated protein 2; the mycophenolate products rely on these pathways for metabolism. When possible, therapeutic drug monitoring can be used to ensure appropriate immunosuppressive exposure while initiating or tapering corticosteroids [7].

Relevant drug interactions have not been noted for the polyclonal/monoclonal antibodies or selective T-cell costimulation blockers. These drugs do, however, pose the risk of pharmacodynamic interactions and additive toxicities.

Since our last update, several new anti-infective medications have been approved for marketing. These include the antibacterial drugs tedizolid, ceftaroline, dalbavancin, oritavancin, ceftolozane–tazobactam, fidaxomicin, and ceftazidime–avibactam. New antiviral agents include antihepaciviral protease inhibitors (telaprevir, boceprevir, simeprevir), polymerase inhibitors, and NS5A inhibitors as well as antiretroviral integrase inhibitors (raltegravir, elvitegravir, dolutegravir). The new azole antifungal agent isavuconazole was also introduced and is marketed as the prodrug isavuconazonium sulfate. New immunosuppressant agents include everolimus and belatacept. The tables highlight only drugs with potential interactions in transplant recipients. Table 4-1 lists the immunosuppressive agents according to their class; Table 4-2 shows common immunosuppressant interactions; and Table 4-3 presents common interactions between anti-infective and immunosuppressant drugs.

TABLE 4-2. Common immunosuppressant drug interactions

Immunosuppressant	Interacts with	Interaction	Clinical effect	Management
Azathioprine (AZA; Imuran)	Allopurinol [9, 10]	AZA is metabolized to 6-MP (active); 6-MP is inactivated by xanthine oxidase (XO); allopurinol inhibits XO	Significant increase in 6-MP exposure; AZA toxicity (i.e., bone marrow suppression)	Reduce AZA dose; will require 66–75 % AZA dose reduction when adding allopurinol
	Aminosalicylates: mesalamine, olsalazine, sulfasalazine [11, 12]	AZA is metabolized to 6-MP (active); 6-MP is inactivated by TPMT; aminosalicylates may inhibit TPMT	Higher risk of bone marrow suppression	Monitor CBC regularly with concomitant use
	Infliximab [13]	Infliximab reduces AZA clearance	Leukopenia	Monitor CBC regularly with concomitant use
	Warfarin [14]	Unknown	Dose-dependent inhibition of warfarin effect	Titrate warfarin; will require ~2.5-fold higher warfarin when adding AZA
	*Cytochrome P-450 3A4 (CYP3A4) and P-glycoprotein drug interactions also apply			

(continued)

TABLE 4-2. (continued)

Immunosuppressant	Interacts with	Interaction	Clinical effect	Management
Cyclosporine (CSA; Neoral, Sandimmune)	Everolimus [15, 16]	Everolimus is a substrate of CYP3A4 and P-gp; CSA inhibits CYP3A4 and P-gp	Concomitant administration of cyclosporine (Neoral) increases EVR AUC and C_{max} by 168 % and 82 %, respectively	EVR dose adjustment may be needed upon initiation or discontinuation of cyclosporine Titrate EVR dose; utilize whole blood trough concentrations
	Micafungin [17]	Micafungin is a mild inhibitor of CYP3A4 in vitro; CSA is a CYP3A4 substrate	Concomitant use results in slightly increased CSA exposure	Titrate CSA; utilize whole blood CSA concentrations
	Mycophenolate mofetil [18, 19]	CSA inhibits enterohepatic recirculation of MPAG	30–50 % reduction in the MPA $AUC_{0–12\ h}$	Note the alteration in MPA exposure when changing concomitant immunosuppression; consider MMF dose adjustments
	Sirolimus [20–22]	Sirolimus is a substrate of CYP3A4 and P-gp; CSA inhibits CYP3A4 and P-gp	Simultaneous administration increases SIR C_{max} and AUC by 116 % and 230 %, respectively	Stagger administration by at least 4 h; note that staggered administration minimizes but does not ameliorate the interaction
			Administering SIR 4 h after CSA increases SIR C_{max} and AUC by 37 % and 80 %, respectively	Titrate SIR; utilize whole blood trough SIR concentrations
	HMG-CoA reductase inhibitors "statins" [23–26]: rosuvastatin, simvastatin > atorvastatin, lovastatin, fluvastatin > pravastatin	Competition for metabolism by CYP3A4; altered statin transport in the liver	Concomitant use results in increased statin exposure; appears more potent with CSA than TAC	Use lower statin doses (i.e., 50 % reduced); watch for myopathies and other statin side effects Use with simvastatin and atorvastatin not recommended
	*Cytochrome P-450 3A4 (CYP3A4) and P-glycoprotein drug interactions also apply			
Everolimus (EVR; Zortress)	Cyclosporine [15, 16]	Everolimus is a substrate of CYP3A4 and P-gp; CSA inhibits CYP3A4 and P-gp	Concomitant administration of cyclosporine (Neoral) increases EVR AUC and C_{max} by 168 % and 82 %, respectively	EVR dose adjustment may be needed upon initiation or discontinuation of cyclosporine Titrate EVR dose; utilize whole blood trough concentrations
	Octreotide [15]	Unknown	Coadministration of EVR with depot octreotide increased C_{min} by 50 %	Note altered octreotide exposure with EVR
Mycophenolate mofetil (MMF; CellCept)	Acyclovir, ganciclovir [27]	The antiviral and MPAG compete for renal tubular secretion; particularly in renal impairment	Risk for increased acyclovir, ganciclovir, and MPAG concentrations	Use combination with caution in renal insufficiency; monitor CBC
	Antacids (i.e., Mg, Al) [28]	Impaired absorption of MMF/MPA	25–33 % reduction in MPA C_{max} and 17–37 % reduction in the MPA $AUC_{0–24\ h}$	Stagger administration by 2–4 h
	Bile acid sequestrants: cholestyramine, colesevelam, colestipol [27, 29]	Drugs that bind bile acids interrupt enterohepatic recirculation	40 % reduction in the MPA $AUC_{0–24\ h}$	Avoid concomitant use
	Oral contraceptives [27]	Unknown	Mean levonorgestrel AUC was decreased by 15 %	Consider additional method of birth control

(continued)

TABLE 4-2. (continued)

Immunosuppressant	Interacts with	Interaction	Clinical effect	Management
	Cyclosporine [18, 19]	CSA inhibits enterohepatic recirculation of MPAG	30–50 % reduction in the MPA $AUC_{0-12\,h}$	Note the alteration in MPA exposure when changing concomitant immunosuppression; consider MMF dose adjustments
	Ganciclovir	See acyclovir		
	Nevirapine [30]	Competition for and/or altered enterohepatic recycling	Slight but significant reduction in nevirapine exposure; unknown effect on MPA	No recommendations have been made
	Proton pump inhibitors [27, 31]	Decreased solubility of MPA at increased gastric pH	MPA C_{max} reduced by 30–70 %, AUC reduced by 25–35 %	Clinical significance unknown Use with caution
	Rifampin [32]	Unknown	Major reduction in MPA $AUC_{0-12\,h}$	Consider MPA drug monitoring while on rifampin
	Sevelamer [33]	Impaired absorption of MMF/MPA	30 % and 25 % reduction in MPA C_{max} and AUC, respectively	Stagger administration by 2 h
Mycophenolic acid (MPA; Myfortic)	Acyclovir, ganciclovir [34]	The antiviral and MPAG compete for renal tubular secretion; particularly in renal impairment	Risk for increased acyclovir, ganciclovir, and MPAG concentrations	Use combination with caution in renal insufficiency; monitor CBC
	Antacids (i.e., Al, Mg) [34]	Antacids decrease MPA absorption	25 % and 37 % reduction in MPA C_{max} and AUC, respectively	Avoid concomitant administration
	Bile acid sequestrants: cholestyramine, colesevelam, colestipol [34]	Drugs that bind bile acids interrupt enterohepatic recirculation	Reduced MPA exposure	Avoid concomitant administration
	Oral contraceptives [34]	Unknown; this interaction is assumed from the MMF experience	Mean levonorgestrel AUC was decreased by 15 %	Consider additional method of birth control
	Cyclosporine [35]	CSA inhibits enterohepatic recirculation of MPAG	20–30 % decrease in the bioavailability and a significant reduction in MPA $AUC_{0-24\,h}$	MPA dose requirements may be higher when used with CSA
	Ganciclovir	See acyclovir		
	*Cytochrome P-450 3A4 (CYP3A4) and P-glycoprotein drug interactions also apply			
Sirolimus (SIR; Rapamune)	Cyclosporine [20–22]	Sirolimus is a substrate of CYP3A4 and P-gp; CSA inhibits CYP3A4 and P-gp	Simultaneous administration increases SIR C_{max} and AUC by 116 % and 230 %, respectively	Stagger administration by at least 4 h; note that staggered administration minimizes but does not ameliorate the interaction
			Administering SIR 4 h after CSA increases SIR C_{max} and AUC by 37 % and 80 %, respectively	Titrate SIR; utilize whole blood trough SIR concentrations
	Micafungin [36]	Unknown	SIR AUC is increased by 21 %; no effect on SIR C_{max}	Consider therapeutic alternatives or titrate SIR doses per whole blood trough SIR concentrations
	*Cytochrome P-450 3A4 and P-glycoprotein drug interactions also apply			

(continued)

TABLE 4-2. (continued)

Immunosuppressant	Interacts with	Interaction	Clinical effect	Management
Tacrolimus (TAC; Prograf, Astagraf XL)	HMG-CoA reductase inhibitors "statins": simvastatin, atorvastatin, lovastatin, fluvastatin, pravastatin, rosuvastatin [23, 26]	Competition for metabolism by CYP3A4; altered statin transport in the liver	Concomitant use results in increased statin exposure; appears more potent with CSA than TAC	Use lower statin doses (i.e., 50% reduced); watch for myopathies and other statin side effects
Cytochrome P-450 3A4 (CYP3A4) and P-glycoprotein (P-gp) drug interactions	Anticonvulsants: carbamazepine, oxcarbazepine, phenobarbital, phenytoin [15, 20, 37, 38]	Induction of CYP3A4-mediated CSA/TAC/SIR/EVR metabolism	Decrease in plasma CSA/TAC/SIR/EVR concentrations	Titrate CSA/TAC/SIR/EVR dose; utilize CSA/TAC/SIR/EVR whole blood concentrations Consider therapeutic alternatives (i.e., valproic acid, lamotrigine, gabapentin)
	Rifampin [15, 20, 39, 40]			Titrate CSA/TAC/SIR/EVR dose; utilize CSA/TAC/SIR/EVR whole blood concentrations Consider rifabutin if appropriate [41]
	St. John's wort [42, 43]		Unpredictable and varying decrease in CSA/TAC/SIR/EVR concentrations	Avoid concomitant use
	Amiodarone [44] Danazol [45] Nefazodone [46]	Inhibition of CYP3A4-mediated CSA/TAC/SIR/EVR metabolism	Increase in CSA/TAC/SIR/EVR concentrations	Titrate CSA/TAC/SIR/EVR dose; utilize CSA/TAC/SIR/EVR whole blood concentrations
	Grapefruit juice [47]		Unpredictable and varying increase in CSA/TAC/SIR/EVR concentrations	Avoid concomitant use
	Macrolide antibiotics: clarithromycin [48–50], erythromycin [51–53], telithromycin		Significant increase in CSA/TAC/SIR/EVR concentrations	Avoid concomitant use whenever possible; consider azithromycin [54] If coadministration is necessary, empirically reduce CSA/TAC/SIR/EVR doses, monitor CSA/TAC/SIR/EVR whole blood concentrations; titrate CSA/TAC/SIR/EVR dose
	Nondihydropyridine calcium channel blockers: diltiazem [55–57] > verapamil [15, 20, 57]		Increase in CSA/TAC/SIR/EVR concentrations; appears to be more potent with diltiazem versus verapamil	Titrate CSA/TAC/SIR/EVR dose; utilize CSA/TAC/SIR/EVR whole blood concentrations
	Anti-HIV protease inhibitors [58]: amprenavir, atazanavir, darunavir, fosamprenavir, indinavir, lopinavir [59, 60], nelfinavir, ritonavir [59, 60], saquinavir Anti-HCV protease inhibitors [61]: boceprevir, telaprevir		Significant increase in CSA/TAC/SIR/EVR concentrations	When initiating CSA/TAC/SIR/EVR, use low doses and utilize whole blood concentrations to determine the dosing interval; may need to utilize liquid formulations to achieve small oral doses Titrate CSA/TAC/SIR/EVR dose and/or interval; utilize CSA/TAC/SIR/EVR whole blood concentrations

(continued)

TABLE 4-2. (continued)

Immunosuppressant	Interacts with	Interaction	Clinical effect	Management
Azole antifungals: itraconazole [62, 63], posaconazole [64, 65], voriconazole [51, 66], isavuconazole [67] > fluconazole [68, 69], ketoconazole [20] > clotrimazole [70, 71]			For ketoconazole, fluconazole, and clotrimazole: titrate CSA/TAC/SIR/EVR dose and/or interval; utilize CSA/TAC/SIR/EVR whole blood concentrations For voriconazole, posaconazole, and itraconazole: empirically reduce CSA/TAC/SIR/EVR doses, monitor CSA/TAC/SIR/EVR whole blood concentrations; titrate CSA/TAC/SIR/EVR dose For voriconazole, ketoconazole, and itraconazole: note that concomitant use with EVR is not recommended [15] For voriconazole and posaconazole: note that concomitant use with SIR is contraindicated [72, 73] but safe coadministration has been reported [64, 74] if SIR doses are cut by at least 50 % before initiating voriconazole or posaconazole; titrate per SIR whole blood trough concentrations Clotrimazole troches can more than double TAC concentrations due to inhibition of intestinal CYP3A4 and P-gp	

Table 4-3. Common interactions between anti-infective agents and immunosuppressive agents

Anti-infective category	Anti-infective agent/class	Immunosuppressive agent or group of agents	Interaction	Clinical effect	Management
Antifungal	Polyene antifungal: amphotericin B formulations	CSA/TAC/SIR/EVR	Additive nephrotoxicity	Increases risk of kidney injury	Monitor CSA/TAC/SIR/EVR concentration and kidney function
Antifungal	Azole antifungals: itraconazole [62, 63], posaconazole [64, 65], voriconazole [51, 66], isavuconazole [67] > fluconazole [68, 69], ketoconazole [20] > clotrimazole [70, 71]	CSA/TAC/SIR/EVR	Inhibition of CYP3A4	Increases CSA, TAC, SIR, and EVR levels in varying amounts	For ketoconazole, fluconazole, and clotrimazole: titrate CSA/TAC/SIR/EVR dose and/or interval; utilize CSA/TAC/SIR/EVR whole blood concentrations For voriconazole, posaconazole, and itraconazole: empirically reduce CSA/TAC/SIR/EVR doses, monitor CSA/TAC/SIR/EVR whole blood concentrations; titrate CSA/TAC/SIR/EVR dose For voriconazole, ketoconazole, and itraconazole: note that concomitant use with EVR is not recommended [15] For voriconazole and posaconazole: note that concomitant use with SIR is contraindicated [72, 73] but safe coadministration has been reported [64, 74] if SIR doses are cut by at least 50 % before initiating voriconazole or posaconazole; titrate per SIR whole blood trough concentration Clotrimazole troches can more than double TAC concentrations due to inhibition of intestinal CYP3A4 and P-gp
		TAC	Additive QTc prolongation	Increases risk of torsade de pointes	Monitor electrocardiogram for QTc prolongation
Antifungal	Anidulafungin/echinocandin	CSA	No significant interaction		
Antifungal	Caspofungin/echinocandin [77–80]	CSA	increase LFTs		Likely not clinically relevant
Antifungal	Micafungin/echinocandin [17]	CSA	Micafungin is a mild inhibitor of CYP3A4 in vitro; CSA is a CYP3A4 substrate	Concomitant use results in slightly increased CSA exposure	Titrate CSA; utilize whole blood CSA concentrations
Antifungal	Micafungin/echinocandin [36]	SIR	Unknown	SIR AUC is increased by 21 %; no effect on SIR C_{max}	Titrate SIR; utilize whole blood SIR concentrations
Antibacterial	Aminoglycosides [81]	CSA/TAC/SIR/EVR	Additive nephrotoxicity	Increases risk of kidney injury	Monitor CSA/TAC/SIR/EVR concentration and kidney function

(continued)

TABLE 4-3. (continued)

Anti-infective category	Anti-infective agent/class	Immunosuppressive agent or group of agents	Interaction	Clinical effect	Management
Antibacterial	Chloramphenicol [82–85]	CSA	Potential inhibition of CYP3A4 and 2C9 (rapid onset)	Increases CSA concentration by about 50%	Monitor CSA concentration and kidney function
Antibacterial	Quinolone antibiotics [37]: ciprofloxacin, ofloxacin, levofloxacin, moxifloxacin	CSA	Inhibition of CYP3A4	Increases CSA concentration	Monitor CSA concentration
Antibacterial	Quinolone antibiotics [86]: ciprofloxacin ofloxacin levofloxacin moxifloxacin	TAC	Inhibition of CYP3A4	Increases TAC concentration	Likely not clinically relevant; monitor TAC concentration
			Additive QTc prolongation	Increases risk of torsade de pointes	Monitor electrocardiogram for QTc prolongation
Antibacterial	Quinolone antibiotics [20]: ciprofloxacin, ofloxacin, levofloxacin, moxifloxacin	SIR	Inhibition of CYP3A4	Increases SIR concentration	Uncertain clinical significance; monitor SIR concentration
Antibacterial	Linezolid, tedizolid [87]	AZA/MMF	Additive bone marrow suppression	Increased risk of thrombocytopenia, leukopenia, and anemia	Monitor CBC and use limited course or select alternatives
Antibacterial	Macrolide antibiotics: clarithromycin [48–50], erythromycin [51–53], telithromycin, azithromycin	CSA/TAC/SIR/EVR	Inhibition of CYP3A4	Significant increase in CSA/TAC/SIR concentrations	Avoid concomitant use whenever possible; consider azithromycin [54] If coadministration is necessary, empirically reduce CSA/TAC/SIR/EVR doses, monitor CSA/TAC/SIR/EVR whole blood concentrations; titrate CSA/TAC/SIR/EVR dose
		TAC	Additive QTc prolongation	Increases risk of torsade de pointes	Monitor electrocardiogram for QTc prolongation
Antibacterial	Metronidazole [37, 88]	CSA/TAC	Inhibition of CYP3A4	Increases CSA/TAC concentration	Monitor CSA/TAC concentration
Antibacterial	Nafcillin [89]	CSA	Unknown mechanism	Increases CSA concentration	Monitor CSA concentration
Antibacterial	Rifampin [32]	MMF	Unknown	Major reduction in MPA $AUC_{0-12\ h}$	Consider MPA drug monitoring while on rifampin [90]
Antibacterial	Rifampin/rifabutin	MMF	Induction of the uridine diphosphate glucuronosyl transferase in the kidney, liver, and intestines		
Antibacterial	Rifampin [15, 20, 39, 40]	CSA/TAC/SIR/EVR	Induction of CYP3A4-mediated CSA/TAC/SIR/EVR metabolism	Decrease in plasma CSA/TAC/SIR/EVR concentrations	Titrate CSA/TAC/SIR/EVR dose; utilize CSA/TAC/SIR/EVR whole blood concentrations Consider rifabutin if appropriate [41]
Antihepaciviral	Protease inhibitors [61]: boceprevir, telaprevir	CSA/TAC/SIR/EVR	CYP3A4 inhibition	Significant increase in CSA/TAC/SIR/EVR concentrations	When initiating CSA/TAC/SIR/EVR, use low doses, and utilize whole blood concentrations to determine the dosing interval; may need to utilize liquid formulations to achieve small oral doses Titrate CSA/TAC/SIR/EVR dose and/or interval; utilize CSA/TAC/SIR/EVR whole blood concentration

Antihepaciviral	Protease inhibitor [91, 92]: simeprevir	CSA	Possible intestinal CYP3A4/P-gp inhibition by simeprevir Inhibition of CYP3A4 and P-gp by CSA	Increase in both simeprevir and CSA concentrations	Monitor CSA whole blood concentration Monitor for increased side effects of simeprevir or avoid combination
		TAC	Unknown	May decrease TAC concentrations	Minimal clinical significance Monitor TAC whole blood concentration
Antiviral	Acyclovir, ganciclovir [27, 34]	MMF	The antiviral and MPAG compete for renal tubular secretion; particularly in renal impairment	Risk for increased acyclovir, ganciclovir, and MPAG concentrations (evidence of increased antiviral levels)	Use combination with caution in renal insufficiency; monitor CBC
Antiviral	Foscarnet [93]	CSA/TAC/SIR/EVR	Additive nephrotoxicity	Increases risk of kidney injury	Monitor CSA/TAC/SIR/EVR concentration and kidney function
Antiretroviral	NNRTI: efavirenz, etravirine, nevirapine [15, 58, 94, 95]	CSA/TAC/SIR/EVR	Induction of CYP3A4-mediated CSA/TAC/SIR/EVR metabolism	Decrease in plasma CSA/TAC/SIR concentrations	Titrate CSA/TAC/SIR/EVR dose; utilize CSA/TAC/SIR/EVR whole blood concentrations
Antiretroviral	Nevirapine [30]	MMF	Competition for and/or altered enterohepatic recycling	Slight but significant reduction in nevirapine exposure; unknown effect on MPA	No recommendations have been made
Antiretroviral	Anti-HIV protease inhibitors [58]: amprenavir, atazanavir, darunavir, fosamprenavir, indinavir, lopinavir [59, 60], nelfinavir, ritonavir [59, 60], saquinavir	CSA/TAC/SIR/EVR	CYP3A4 inhibition	Significant increase in CSA/TAC/SIR/EVR concentrations	When initiating CSA/TAC/SIR/EVR, use low doses, and utilize whole blood concentrations to determine the dosing interval; may need to utilize liquid formulations to achieve small oral doses Titrate CSA/TAC/SIR/EVR dose and/or interval; utilize CSA/TAC/SIR/EVR whole blood concentration

Efavirenz: induction increases (more rapid CL) TAC and CSA and prednisolone.

Abbreviations: *CSA* cyclosporine (Neoral, Sandimmune), *TAC* tacrolimus (Prograf, Astagraf XL), *SIR* sirolimus (Rapamune), *EVR* everolimus (Zortress), *MMF* mycophenolate mofetil (CellCept), *AZA* azathioprine (Imuran), *HIV* human immunodeficiency virus, *HCV* hepatitis C virus.

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5
Diagnostic Testing: General Principles

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5.1 Introduction

Solid organ (SOT) and hematopoietic stem cell transplantation (HSCT) are processes that result in complex interactions between the donor and the recipient and require the use of immunosuppressive medications to induce immune tolerance. Infectious complications are an unfortunate consequence of transplantation and careful assessment before, during, and after transplantation is necessary to prevent significant morbidity and mortality. Recognition of infection is often more difficult in transplant recipients due to the necessary suppression of immune function and as a result, the use of diagnostic assays for the identification of infection is of increased importance [1]. When considering optimal diagnostic testing strategies in transplant recipients, the infectious disease history prior to transplantation in both the donor and recipient, factors related to the transplantation itself (nosocomial processes or procedure-related complications), and issues unique to the use of immunosuppression to prevent organ rejection or graft vs. host disease (GVHD) must be considered [1–3].

Having an understanding of the testing used in the diagnosis of transplant-related infectious diseases, including the strengths and pitfalls of each test, is vital in care of the transplant recipient. The goal of this chapter is to provide an overview of the use of diagnostic tests in the care of the transplant patient. More specifically, testing strategies for both before and after SOT and HSCT will be discussed with a focus on appropriate use for risk stratification and clinical decision-making in this population.

5.2 Diagnostic Testing Prior to Transplantation

Given the potential for both transmission and reactivation of infections after transplantation, pretransplantation screening of potential donors and recipients is performed prior to the procedure. This process allows for the identification of any conditions which might preclude either the donor or the recipient from taking part in the transplantation and to identify and treat any active infections prior to the initiation of immunosuppressive therapy [4]. Pretransplantation screening also allows a physician to better define the risk of infection and to develop strategies for preventing infection through diagnostic monitoring, the use of prophylaxis, or immunization prior to transplantation [4]. Diagnostic testing for both active and latent infections in the pretransplantation setting is an integral part of the pretransplantation evaluation and is often tailored to the organ or cells to be transplanted as well as the epidemiology or risk factors of the donor and recipient.

5.3 Pretransplant Testing in Solid Organ Transplantation

5.3.1 Donor Screening: Living Donors–Routine Testing

The use of organs obtained from living donors provides a unique opportunity to assess the donor for the presence of active and quiescent infectious diseases that could potentially be transmitted to the recipient via the transplanted organ. For living donors, pretransplantation diagnostic testing for the following infections is required: cytomegalovirus (CMV), Epstein-Barr Virus (EBV), human immunodeficiency virus (HIV), Hepatitis B (HBV), Hepatitis C (HCV), and syphilis [5, 6] (Table 5-1). All of these tests must be performed using FDA-licensed, -approved, or cleared tests in a CLIA-certified laboratory or one that meets equal conditions as determined by the Centers for Medicare and Medicaid [5].

5.3.1.1 Cytomegalovirus and Epstein-Barr Virus

The transmission and acquisition of CMV from donor to recipient may result in significant morbidity. Although prior infection with CMV in the donor and/or recipient is not a

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TABLE 5-1. Pretransplant diagnostic testing in solid organ transplant donors and recipients

	Test	Donor		Recipient	
Organism		Routine	Optional	Routine	Optional
CMV	IgG	X		X	
EBV	VCA IgG	X		X	
VZV	IgG[a]			X	
HIV-I/II	Ag/AB[b,c]	X		X	
	NAT		X[d]		X[d]
HAV	IgG			X	
HBV	sAg	X		X	
	anti-HBc (IgM and IgG)	X		X	
	anti-HBs			X	
	NAT		X[e]		
HCV	Anti-HCV[f]	X		X	
	NAT	X		X	
HTLV-I/-II	IgG[g]		X		X
WNV	NAT[h]		X		
MMR	IgG			X	
Syphilis	TP-EIA[i]	X		X	
TB	TST or IGRA[j]	X		X	
T. cruzi	IgG		X		X
Strongyloides	IgG[k,l]		X		X
Toxoplasma	IgG	X[m]		X[m]	
Coccidioides	IgG[n]		X		X
Histoplasma	IgG[o,p]		X		

[a]ELISA antibody assay preferred given its higher specificity for detection [29, 30].
[b]If Ag/AB testis positive, HIV-1/-II differentiation assay is performed to differentiate HIV-I and HIV-II infection [14].
[c]If discrepancies between HIV-I/-II Ag/AB test and HIV-I/-II differentiation assay HIV NAT should be performed [14].
[d]HIV NAT should be performed if potential donor or recipient is identified as high risk for HIV infection [6].
[e]HBV NAT should be performed when anti-HBC alone is positive to assess for occult infection.
[f]Positive anti-HCV with a negative HCV NAT is followed with repeat anti-HCV with another FDA-approved assay [13].
[g]A positive screening test for HTLV-I/II should be confirmed with virus-specific western blot or line immunoassay [25].
[h]WNV IgM testing is not FDA-approved for screening but can be performed to increase the sensitivity of WNV detection [28].
[i]If TP-EIA is positive, RPR is then performed with a positive result confirming the diagnosis. If RPR is negative, TPPA is performed to confirm diagnosis. If TPPA is negative, FTA-ABS is performed to confirm diagnosis. If FTA-ABS is negative, TP-EIA is considered a false positive result [8, 9].
[j]A two-stage TST should be performed in living donors and recipients from endemic regions [4, 19].
[k]ELISA antibody assay preferred for *Strongyloides* given its higher sensitivity and specificity [22, 23].
[l]Stool examination for ova can be performed if antibody assay is unavailable but is not recommended due to its low sensitivity [22].
[m]Routine testing for *Toxoplasma* is only recommended in potential cardiac donors and recipients.
[n]EIA, complement fixation, and immunodiffusion assays available for *Coccidioides* screening [22, 24].
[o]Screening for *Histoplasma* with immunodiffusion or complement assay is recommended [24].
[p]Presence of H precipitin band on immunodiffusion assay of complement fixation titer ≥ 1:32 is diagnostic of active infection [24].

contraindication to transplantation, knowing the serostatus of the donor and recipient is critical in defining the recipient's risk for developing CMV disease and for optimizing risk reduction strategies either through the use of prophylaxis or preemptive monitoring. Donor screening to assess for past exposure to CMV should be performed using assays with high sensitivity and specificity for anti-CMV IgG. Tests that include detection and measurement of CMV IgM should be avoided due to poor specificity [7]. Equivocal anti-CMV IgG results in the potential donor should be interpreted as positive to ensure appropriate prophylactic and monitoring strategies of the recipient in the posttransplant setting [7].

Acquisition of EBV transmitted from donor to recipient may result in acute EBV infection in previously uninfected recipients. Active EBV replication has been associated with the development of posttransplantation lymphoproliferative disorder (PTLD). As EBV induces the production of several different EBV-specific antibodies, testing for past exposure with the viral capsid Antigen IgG (VCA IgG) is the diagnostic study of choice [4]. Nonspecific screening tests for EBV

such as heterophile antibody is not an acceptable strategy as the production of heterophile antibodies typically wane within months to 1 year after infection.

5.3.1.2 Syphilis

Syphilis screening should be performed according to the syphilis testing algorithm described by the Centers for Disease Control (CDC) and begins with an initial enzyme immunoassay treponemal test (TP-EIA) [8, 9]. If TP-EIA is positive, a non-treponemal test such as the rapid plasma regain (RPR) should then be performed with a positive result confirming the diagnosis of syphilis [8, 9]. In the event of a negative non-treponemal antibody test, a second treponemal test such as the *Treponema pallidum* particle agglutination (TPPA) should be performed [8, 9]. If the second treponemal antibody test is negative, a third treponemal antibody test, such as the fluorescent treponemal antibody (FTA-ABS), is then done [8, 9]. If either the second or third treponemal antibody test is positive, a diagnosis of syphilis is made and treatment of the donor should be considered. Negative results of the RPR, TPPA, and FTA-ABS in the setting of a positive TP-EIA indicate either a false positive result or prior resolved infection [8, 9].

5.3.1.3 Testing for Hepatitis C Virus and Human Immunodeficiency Virus

Given the potentially devastating consequences of acquiring HCV and/or HIV infection from a potential organ donor, screening for these viruses is a complex process and depends on the use of both serology and direct virus detection in the blood stream via nucleic acid testing (NAT) for accurate diagnosis. These tests are performed to improve the sensitivity of detection of virus in donors with potential recent infection when seroconversion or detection of antibody production has not yet occurred. Termed the "window period," this period of time is often marked by high levels of circulating antigen or virus prior to the development and detection of a virus-specific antibody response. Therefore, testing for antibodies directed against HCV and HIV during this time period can result in a "false negative" result and can lead to unintended transmission [10]. As positive results for HCV and HIV can have implications for donor eligibility, combining these testing modalities improves the sensitivity of detecting these infections prior to transplantation [5, 6].

5.3.1.3.1. Hepatitis C Virus

Donor screening forHCV is performed using both anti-Hepatitis C antibody (anti-HCV) and a Hepatitis C nucleic acid test (HCV NAT) and must be performed within 28 days of donation [5, 6]. HCV NAT allows for the direct detection of HCV RNA in donor plasma at RNA levels as low as 2.0–9.4 IU/mL [11, 12]. This highly sensitive assay has reduced the preseroconversion window period to approximately 4–6 days from exposure as compared to approximately 60 days with antibody testing alone, greatly increasing the diagnosis of acute HCV infection in potential donors [11]. A positive HCV NAT with or without a positive anti-HCV is indicative of active hepatitis C infection and is considered to be a contraindication to living liver donation [5]. Due to the waxing and waning nature of Hepatitis C viremia, a single negative HCV NAT cannot be used as a stand-alone test to rule out HCV infection and must be paired with anti-HCV Ab testing. A positive anti-HCV in the absence of detectable HCV RNA, however, can indicate either a resolved infection with HCV or a false positive result [11, 13]. If there is concern for a false positive anti-HCV, current CDC guidelines recommend repeat testing for anti-HCV with another Food and Drug Administration (FDA)-approved assay [13]. Although controversy exists around the use of organs other than the liver from a donor with anti-HCV Ab positivity; donation may be considered in cases where the recipient is severely ill or who have prior infection with HCV with appropriate informed consent [4].

5.3.1.3.2. Human Immunodeficiency Virus

Screening for HIV infection should be performed using a fourth-generation antigen/antibody combination immunoassay which detects both HIV-1 and HIV-2 and must be performed within 28 days of donation [5, 6, 14]. This test identifies antibody directed against HIV type 1 and HIV type 2 as well as the presence of HIV-1 p24 antigen, a protein shed into the blood stream during periods of active viral replication. Shedding of p24 antigen typically occurs at high levels shortly after initial HIV infection and may be detectable 7–10 days prior to antibody [15]. Screening with a fourth-generation antigen/antibody combination test results in a significant shortening of the window period prior to seroconversion, increasing the sensitivity of acute HIV detection [14].

If a potential donor is identified as high risk for HIV infection, HIV nucleic acid testing (HIV NAT) should also be performed to increase the sensitivity of diagnosis [6]. HIV NAT is the first detectable biomarker in acute HIV infection, preceding the appearance of p24 antigen by several days [16]. If the initial combination HIV-1/2 antigen/antigen test is positive, supplemental testing with an HIV1/2 differentiation assay should be performed to exclude the possibility of a false positive screening test and to differentiate HIV-1 from HIV-2 infection [14]. Due to the detection of p24 antigen, donors with acute HIV-1 infection may have a reactive HIV1/2 antigen/antibody screening test prior to the development of HIV-1-specific antibody. Therefore, discrepancies between

the results for the initial HIV 1/2 antigen/antibody test and the HIV 1/2 differentiation assay should be resolved with HIV NAT testing [14]. Donors who test positive for HIV are excluded from donation [5, 17].

5.3.1.4 Hepatitis B Virus Screening

For HBV, testing for both Hepatitis B surface antigen (HBsAg) and Hepatitis B core antibody to both IgM and IgG (anti-HBc) should be performed. The presence of these markers is highly sensitive for the detection of active HBV infection [4–6, 18]. Unlike HCV and HIV screening, HBV nucleic acid testing (HBV NAT) is not routinely performed in donors from countries with low endemic rates of HBV infection as the additional benefit of HBV NAT testing appears to be small [19]. In instances when anti-HBc alone is positive, further testing for HBV should be performed with a hepatitis B nucleic acid testing (HBV NAT). The detection of HBV DNA in this setting should be considered diagnostic of active HBV infection. Potential living donors who are positive for HBsAg are excluded from donation [5]. The decision to use an organ from a positive anti-HBc donor regardless of HBV DNA testing is a complex decision often made on a case-by-case basis. Testing must be performed within 28 days of donation [5].

5.3.2 Donor Screening: Deceased Donors-Routine Testing

In the United States, pretransplant diagnostic testing for deceased donors is performed by organ procurement organizations (OPO's) for all transplant programs in a given Donation Service Area (DSA) [5]. As with living donors, all laboratory testing must be completed in CLIA-certified laboratories or in one which meets the same standards as determined by the Centers for Medicare and Medicaid Services using FDA-licensed or -approved tests [5]. In general, deceased donor screening is similar to the approach described for living donors. Testing for CMV, EBV, syphilis, HIV, HBV, HCV is required and is the same as that described above for living donors [5, 6]. In addition, blood cultures and cultures from the harvested organ (i.e., Urine from donor kidney or sputum and/or bronchoalveolar lavage fluid (BAL) from the donor lung) should be performed at the time of transplantation [4, 5]. This is done to assess for active infection as well as colonizing flora which could result in invasive infection after transplantation [4]. In the case of potential deceased heart donation, antibody testing for *Toxoplasma* IgG must be performed given the potential development of this infection from seropositive donors after transplantation [4]. Donor screening for toxoplasmosis in noncardiac donors is not required [4].

5.3.3 Donor Screening: Optional Testing in Donors

Although the rationale for the routine testing detailed above identifies the most common donor-derived pathogens, there are many other less common latent infections that can be transmitted by the donor to the recipient at the time of transplantation. The presence of these latent infections is largely determined by the donor's epidemiologic exposure. A thorough history from the donor or a close family member is essential in determining these epidemiologic risk factors which can then allow for further targeted diagnostic testing. The most common of these pathogens include *Mycobacterium tuberculosis*, *Strongyloides stercoralis*, *Trypanosoma cruzi*, *Histoplasma capsulatum*, *Coccidioides immitis*, Human T lymphotrophic virus-1 (HTLV-1), and West Nile virus.

5.3.3.1 Mycobacterium tuberculosis

Current OPTN policies recommend testing for*Mycobacterium tuberculosis* (TB) in living donors if a potential donor is considered to be at increased risk for latent infection and the American Society of Transplantation recommends testing for TB in all living donors regardless of the donor's individual risk [4, 5]. Testing should be performed using either the Mantoux tuberculin skin test (TST) or an interferon-gamma release assay (IGRA) [4, 5, 20]. A two-stage TST in which a negative TST result is repeated 1–3 weeks later, can be performed in living donors from TB endemic regions [4, 20]. The TST measures a delayed hypersensitivity reaction to the presence of purified TB protein derivative in those infected with TB whereas the IGRA measures the cellular immune response to TB proteins through the release of interferon-gamma (IFN-γ) [21]. Both TST and IGRA have sensitivities ranging between 80 and 90% [21]. IGRA testing does have an increased specificity for the diagnosis of TB infection as it does not cross react with Bacillus Calmette-Guerin (BCG) vaccination as compared to the TST [21]. Neither test seems to be superior for the diagnosis of TB in immunosuppressed individuals [21]. In a living donor, a positive TST or IGRA must be followed by additional testing to assess for active TB infection [4, 21]. While TST cannot be performed in deceased donor blood [22], IGRA testing can be performed, however, the utility of these tests have not been studied in this population and therefore cannot be used solely to determine the overall TB risk [22]. Furthermore, the time constraints associated with potential deceased donation make IGRA testing impractical as results will often not be available by the time the decision for donation is made [4]. Latent TB infection in both living and deceased donors is not a contraindication to donation although active infection in a living or deceased potential donor is a cause for exclusion [4].

5.3.3.2 *Strongyloides stercoralis*

Donor-derived transmission of parasitic infections has been well documented in the posttransplant setting. *Strongyloides stercoralis* is a common parasite found in tropical climates that can live latently within an asymptomatic potential donor [23]. Although rare, donor-derived transmission of this infection can result in significant morbidity and mortality to the recipient [23]. Potential donors with epidemiologic risk factors for *Strongyloides stercoralis* should be screened for this infection prior to donation. The preferred screening method is serology with enzyme-linked immunosorbent assay (ELISA) for IgG antibody as this has a greater sensitivity and specificity for the detection of the organism as compared to other methods [23, 24]. Stool examination for *Strongyloides stercoralis* ova can also be performed but is not recommended given its significantly lower sensitivity [23]. It may be considered when antibody testing cannot be performed. A positive test for *Strongyloides stercoralis* is not a contraindication to donation in potential living donors but would require treatment prior to donation [23]. A positive test in deceased donors is also not a contraindication to donation but would require treatment in the recipient following transplantation.

5.3.3.3 *Trypanosoma cruzi*

Trypanosoma cruzi is another parasitic infection that can be transmitted from donor to recipient at the time of transplantation. Endemic in Central and South America, infected individuals develop chronic infection which is often asymptomatic [23]. Screening of all potential donors who have lived in an endemic region is recommended and is often done with serology testing [23]. There are currently several FDA-cleared tests for the identification of latent infection in potential donors. A positive test for *T. cruzi* is a contraindication to cardiac donation but not for other organ donations [4].

5.3.3.4 *Endemic Fungal Infections*

Donor-derived transmission of endemic fungi such as *Histoplasma capsulatum* and *Coccidioides immitis* is an uncommon occurrence in the posttransplant setting, however, when transmission occurs, morbidity and mortality is high [4, 23]. Due to overall low rates of transmission, routine testing for *Histoplasma* in potential candidates from endemic regions is not recommended [25]. Potential living donors from endemic areas who have a history of histoplasmosis or a pneumonia of unclear etiology within the past 2 years, however, should undergo diagnostic testing [25]. Serologic testing with the use of immunodiffusion assay or complement fixation is recommended with the identification of an H precipitin band on immunodiffusion assay or a complement fixation titer of ≥1:32 being diagnostic of active infection [23, 25]. The identification of an M precipitin band on immunodiffusion or complement fixation titers <1:32 are less specific for active infection and may indicate past infection with a low risk for transmission [23, 25]. Outside the setting of suspected active *Histoplasma* infection, serum or urine *Histoplasma* antigen testing has limited utility and should be avoided as a screening test in the asymptomatic living donor. Potential deceased donors from endemic regions with symptoms suggestive of histoplasmosis should undergo organ examination for signs of infection [25]. If present, tissue for fungal histopathology and culture as well as serum *Histoplasma* serology and antigen assay should be performed [25]. Active disseminated infection in a potential donor is a contraindication to donation, however in deceased donors, identification of this is often made after transplantation [4, 25].

The majority of patients with transmissible *Coccidioides immitis* infection are asymptomatic [23]. Therefore, potential living donors from endemic regions should be screened with serologic assays in combination with chest imaging [25, 26]. Multiple serologic assays are available including enzyme immunoassay (EIA), complement fixation (CF), and immunodiffusion (ID) [25]. Detection of IgG antibody via immunodiffusion has the highest specificity for diagnosis but has a lower sensitivity than other assays particularly early in infection [25]. Potential living donors with a positive serological test should then undergo further evaluation and testing to identify the degree of infection including cultures, imaging, and cerebrospinal fluid (CSF) studies depending on the clinical situation [25]. Active infection in a potential living donor is a contraindication to transplant until treatment has been completed [25]. Potential deceased donors from endemic areas should undergo organ examination followed by cultures, fungal histopathology, and serologic testing. Active known *Coccidioides* infection in a potential deceased donor is a contraindication to transplant, however, diagnosis is often made after transplantation [27].

5.3.3.5 *Human T-Cell Lymphocytic Virus-1*

Human T-cell lymphocytic virus-1 is endemic in parts of the Caribbean, Japan, and West Africa. Donor-derived transmission of HTLV-1 is a rare but potentially serious complication in SOT. Due to the overall low seroprevalence of this virus in the United States, routine testing of potential donors is no longer performed [4]. Optional screening of potential donors at risk for asymptomatic HTLV-I infected, however, can be performed using FDA-approved screening tests for HTLV-1/2 [26, 28]. These are combined serologic screening tests for both HTLV-I and HTLV-II [26, 28]. One limitation to testing is that the currently available assays are unable to

distinguish between HTLV-I and HTLV-II infection [26]. As HTLV-II has never been determined to cause human disease, being able to distinguish between these two entities is clinically important [26]. Therefore, a positive initial assay should always be confirmed by a virus-specific western blot or line immunoassay [26]. The presence of known HTLV-I in a potential donor results in exclusion from donation [4].

5.3.3.6 West Nile Virus

Donor-derived transmission of West Nile Virus (WNV) has been documented in SOT. Targeted testing of potential living donors in locations of WNV activity and during time periods when transmission is thought to be high can be performed [29]. WNV nucleic acid testing (WNV NAT) is the preferred diagnostic testing strategy and ideally should be performed using an FDA-approved WNV NAT, within 2 weeks of donation [28, 29]. Serologic testing for IgM to WNV is also available but is not an FDA-approved screening test as it has not been studied for this indication [29]. It can be considered as a supplemental test to WNV NAT to increase the sensitivity of WNV detection [29]. Overall, the false positive rate of WNV NAT is low, however, if a false positive is suspected, repeat WNV NAT using another NAT can be performed [29]. Potential living donors with a positive test for WNV should be excluded from donation [4]. Potential deceased donors with symptoms suggestive of WNV should also undergo NAT testing and if there is clinical suspicion of infection, they should be excluded from organ donation [4].

5.3.4 Recipient Screening: Routine Testing

For potential SOT recipients, routine pretransplant diagnostic testing for the following infections is recommended: CMV, EBV, HIV, HBV, HCV, syphilis, TB, varicella (VZV), hepatitis A (HAV), measles, mumps, and rubella (MMR) [4]. Testing strategies for CMV, EBV, HCV, syphilis, and TB are the same as those described above for potential donors. For HBV screening in the potential recipient, anti-HBs testing in addition to HBsAg and anti-HBc to assess for potential immunity is recommended. Potential recipients who are seronegative for HBV should undergo vaccination prior to transplant [4]. VZV screening should be performed using standard serological techniques for the detection of IgG antibody to determine if vaccination is needed prior to transplantation. It should be noted, however, that these assays have a low sensitivity for the detection of vaccine-induced immunity and can lead to false negative reports in those previously vaccinated [30]. ELISA-based assays are preferred given their higher specificity for the detection VZV immunity in order to prevent false positive results [30, 31]. Diagnostic testing for HAV and MMR should be performed using standard commercially available serologic assays in all potential recipients to assess for immunity [4]. Seronegative individuals should receive vaccination prior to transplantation if there are no contraindications [4]. In all individuals being considered for heart transplantation, testing for anti-*Toxoplasma IgG* must be performed given the potential development of this infection after transplantation [4]. Serologic methods as described above in deceased donor screening are recommended. Finally, screening of the potential SOT recipient for active infection and multi-drug-resistant colonizing flora through the use of standard culture techniques or NAT is often done prior to transplantation [4].

The development of CMV infection is one of the most common infectious complications of transplantation. As mentioned in the section on donor screening, testing is performed using anti-CMV IgG due to its improved specificity for detection of latent infection [7]. Assays that measure CMV IgM should not be used due to the poor specificity. Careful interpretation of anti-CMV IgG results is required as false positive results have been reported in individuals with multiple blood transfusions or in children less than 1 year of age from passive transfer of anti-CMV IgG antibodies [7, 32]. Whenever possible, a pretransfusion blood sample is best for testing [7]. If pretransfusion testing is not possible and/or equivocal results persist in the potential recipient, the result should be interpreted so as to ascribe the highest CMV risk to that individual based on the donor's CMV serostatus [7]. In children less than 1 year of age, CMV culture or NAT of numerous mucosal sites should be performed to help decipher any equivocal findings [7].

5.3.5 Recipient Screening: Optional Testing

Individuals at risk for *Strongyloides stercoralis*, *Toxoplasma gondii*, *Trypanosoma cruzi*, HTLV-1, and *Coccidioides immitis* should be screened as previously described [4]. Pretransplantation screening for *Histoplasma capsulatum*, however, is not recommended given the tests low sensitivity for the detection of latent infection in potential recipients [4]. Diagnostic testing for HTLV-I can also be performed and follows the recommendations previously described above [4].

5.4 Pretransplant Testing in Hematopoietic Stem Cell Transplant

5.4.1 Donor Screening: Routine Testing

The Food and Drug Administration (FDA) currently requires that all potential HSCT donors be screened for CMV, HIV, HBV, HCV, syphilis, HTLV-I/II, and WNV [33]. Testing for HIV, HBV, HCV, syphilis, and HTLV-I/II is also supported

TABLE 5-2. Pretransplant diagnostic testing in hematopoetic stem cell transplant donors and recipients

		Donor		Recipient	
Organism	Test	Routine	Optional	Routine	Optional
CMV	IgG	X		X	
EBV	VCA IgG	X		X	
VZV	IgG[a]			X	
HSV-I/II	IgG			X	
HIV-I/II	AB or Ag/AB	X		X	
	NAT	X		X	
HBV	sAg	X		X	
	anti-HBc (IgM and IgG)	X		X	
	anti-HBs			X	
	NAT	X			X[b]
HCV	Anti-HCV	X		X	
	NAT	X		X	
HTLV-I/-II	IgG	X		X	
WNV	NAT[c]	X		X	
Syphilis	RPR or TP-EIA	X[d]		X	
TB	TST or IGRA[e]				X
T. cruzi	IgG		X		X
Strongyloides	IgG[f,g]		X		X
Toxoplasma	IgG		X		X
Malaria	Malaria rapid antigen[h]				X
Ehrlichia	IgG[i]		X		
Anaplasma	IgG[i]		X		
Coxiella	IgG[j]		X		
Babesia	Blood smear light microscopy or PCR		X		
Rickettsia rickettsii	IgG[j]		X		

[a]ELISA antibody assay preferred given its higher specificity for detection [29, 30].
[b]If HBsAg or anti-HBc is positive, HBV NAT should performed.
[c]WNV IgM testing is not FDA-approved for screening but can be performed to increase the sensitivity of WNV detection [28].
[d]Potential donors with a positive RPR must have a TP-EIA performed to confirm the diagnosis [32].
[e]A two-stage TST can be performed in recipients from endemic regions [4, 19].
[f]ELISA antibody assay preferred for *Strongyloides* given its higher sensitivity and specificity [22, 23].
[g]Stool examination for ova can be performed if antibody assay is unavailable but is not recommended due to its low sensitivity [22].
[h]A negative malaria rapid antigen diagnostic test should be confirmed by thick and thin blood smear examination [40, 41].
[i]For *Ehrlichia* and *Anaplasma,* an immunofluorescence assay is recommended with a fourfold increase in titer being diagnostic [37].
[j]Serology for *Coxiella* and *Rickettsia* using indirect immunofluorescence antibody is preferred [37, 38].

by the American Association of Blood Banks (AABB) [34] (Table 5-2). For HIV, either a third generation test for HIV-1/2 antibody or a fourth generation antigen/antibody combination immunoassay for HIV-1/2 as well as HIV NAT is recommended [33, 34]. For CMV, HCV, and HTLV-I/II the diagnostic methods described in the section on SOT donor screening should be used [33]. For HBV, the FDA requires anti-HBc and HBsAg testing whereas the AABB also recommends HBV NAT testing to assess for HBV infection [33, 34]. Detection of HBV DNA by NAT is diagnostic for HBV infection although a single negative (undetectable) NAT result is not sufficient to rule out this infection. Potential donors who test positive for HIV or HTLV-I/II on initial screening tests are excluded from donation [33]. Potential donors who test positive for HBV (a positive HBsAg, anti-HBc or HBV NAT) and HCV are generally excluded from donation although in some situations exceptions may be made [33]. Confirmatory testing for these viruses can and should be performed in relation to the donor but will not change the outcome of donor ineligibility [33].

CMV seropositivity is not a contraindication to donation but does inform prophylaxis or monitoring strategies in the recipient following transplantation [33]. For syphilis screening, either a non-treponemal antibody test such as the RPR or a treponemal antibody test such as the TP-EIA can be performed [33]. Potential donors with a positive treponemal-specific antibody test are excluded from donation whereas due to the low specificity of non-treponemal antibody assays,

those with an initial positive non-treponemal antibody test but a negative confirmatory treponemal antibody test are not excluded [33]. Testing must be performed within 30 days of donation for stem cell and bone marrow transplantation and within 7 days for umbilical cord blood transplantation [2]. For WNV, WNV NAT as described in the section on screening of SOT donors should be performed on all potential donors [29]. Potential donors who test positive for WNV should be deferred from donation for 120 days [35]. Although not required by the FDA, screening for EBV infection with the use of VCA IgG is often performed to assess for risk of posttransplantation proliferative disorders.

5.4.2 Donor Screening: Optional Testing

As with SOT, transmission of other less common latent infections from a potential HSCT donor can have significant consequences in the recipient posttransplantation. Pretransplantation screening for these infections includes a thorough history to identify epidemiological risk factors in the potential donor. If risk factors are identified, further diagnostic testing is then performed.

Similar to SOT, *Trypanosoma cruzii* transmission has been described in HSCT donation and therefore, serologic testing using an FDA-approved assay should be performed in potential donors from endemic regions [2, 36]. Potential donors who are repeatedly seropositive on a screening assay are deferred from donation regardless of the results of confirmatory testing [36].

Potential donors with symptoms suggestive of active *Mycobacterium tuberculosis* should be evaluated fully for evidence of infection and excluded from donation if a diagnosis of active TB is made [2]. Latent TB in a potential donor, however, is not a contraindication to donation as the risk of transmission is minimal [2]. Therefore, routine screening of potential donors with risk factors for latent TB is not recommended [2].

Pretransplantation screening of potential donors for malaria is recommended in those who have resided or travelled to an endemic area [2]. Initial screening involves a careful history. Potential donors with a history of travel to an endemic area should be deferred from donation for 1 year from return and those who have resided in a malaria endemic region should be deferred from donation for a 3-year period [2]. Diagnostic testing for asymptomatic malaria infection in potential donors with potential exposure is not recommended as the likelihood of detecting parasitemia during dormant disease is exceedingly low [2, 34].

Pretransplantation screening for *Toxoplasma gondii* can be performed in potential donors with risk factors as asymptomatic transmission to recipients has been reported [2, 37]. Antibody testing as described in the screening of SOT donor section is the preferred diagnostic tool. Potential donors with acute infection should be deferred from donation, however, past or latent infection is not a contraindication to donation [2].

Although no current FDA guideline exists, testing for tick borne illnesses such as *Ehrlichia*, *Anaplasma*, *Coxiella*, *Babesia*, and *Rickettsia rickettsii* can also be considered in potential donors residing in endemic areas. The diagnosis of Babesiosis can be made either with detection of the parasite directly on thick and thin blood smear examination or through nucleic acid amplification of the *Babesia* 18s rRNA gene through polymerase chain reaction (*Babesia* PCR) [38]. Potential donors with either evidence of *Babesia* or a past history of infection should be deferred per AAAB recommendations. NAT is the most sensitive way to make the diagnosis of *Ehrlichia* and *Anaplasma*; however, serologic testing using immunofluorescence assay (IFA) remains the test of choice with a fourfold increase in IgG antibody indicating active infection [38]. For *Coxiella,* serology through indirect immunofluorescence methods is the diagnostic study of choice [38]. For *Rickettsia rickettsii* or Rocky Mountain Spotted Fever (RMSF) diagnosis is often made based on clinical features and epidemiology [39]. Serologic testing using indirect immunofluorescence antibody (IFA) can be performed and it has been shown to have a 94–100 % sensitivity for the diagnosis of RMSF after 2 weeks of onset of symptoms [39]. Sequential serologic testing looking for acute and convalescent titers is the recommended approach [39]. There is no formal recommendation from the FDA or the AABB regarding deferment of potential donors with active infection with *Coxiella*, *Ehrlichia*, *Anaplasma*, or *Rickettsia rickettsii* although most would recommend deferment until resolution of illness [40].

5.4.3 Recipient Screening: Routine Testing

Pretransplantation screening of potential HSCT recipients for CMV, EBV, Herpes Simplex Virus (HSV), VZV, HBV, HCV, HIV, and syphilis is recommended [2, 37]. Testing for WNV and HTLV-I/II is also often routinely performed by blood banks. Testing for CMV, EBV, HCV, HIV, syphilis, WNV, and HTLV-I/II should follow the testing methods outlined in the section on diagnostic methods for SOT donors. Testing for VZV should follow the methods outlined in the section of diagnostic methods for SOT recipients. For HBV screening, HBsAg, anti-HBc, and anti-HBs should be performed [2]. If a recipient tests positive for anti-HBc or HBsAg, nucleic acid testing (HBV NAT) should then be done [2]. For HSV, IgG serology for HSV should be performed using standard serologic methods; type specific anti-HSV IgG is not required [2]. Positive serologies for any of the above latent infections are not a contraindication to HSCT but do inform the physician regarding potential reactivation risk in the recipient and allow for the implementation of appropriate monitoring or prophylactic strategies.

5.4.4 Recipient Screening: Optional Testing

Similar to SOT recipients, potential HSCT recipients may also harbor other latent infections at the time of transplantation depending on their epidemiologic exposure risk. For TB, routine diagnostic testing of all potential recipients for *Mycobacterium tuberculosis* is controversial. Those with risk factors for TB, should be screened using the diagnostic methods previously described in the SOT section [2]. Transplantation of a potential recipient with active TB should be deferred until the infection is controlled whereas latent infection should not delay transplantation [2].

Reactivation of parasitic infections has been well documented in HSCT recipients. For example, reactivation of *Toxoplasma gondii*, *Strongyloides stercoralis*, and *Trypanosoma cruzii* have all been described. For *Toxoplasma*, potential recipients with risk factors for latent infection should be screened using IgG antibody testing [2, 37]. For *Strongyloides*, screening using antibody testing as described in the section on SOT is the preferred diagnostic method [2]. Stool examination for ova and parasite is not recommended due to its low sensitivity but can be performed if antibody testing is not available [2]. In that setting, at least three stool samples should be evaluated to increase the sensitivity of detection [2, 37]. Testing for *Trypanosoma* follows those diagnostic methods previously outlined in the SOT section.

Pretransplantation screening of potential recipients for malaria is recommended in those who have resided or travelled to an endemic area [2]. Initial diagnosis should be made through the use of a Malaria Rapid antigen diagnostic test which detects the presence of both a *Plasmodium falciparum*-specific antigen and a pan malaria antigen found in all malarial species in whole blood of individuals with detectable malaria parasitemia [41]. The only currently FDA-approved Malaria Rapid Antigen diagnostic test has a 96 % sensitivity for the detection of *P. falciparum* and an 84 % sensitivity for non-*P. falciparum* infections [41]. Given the potential for false negative results, especially for the detection of non-*P. falciparum* species, the CDC currently recommends that all negative Malaria Rapid antigen diagnostic tests be confirmed by thick and thin blood smear examination [42]. Potential recipients who test positive for malaria should be treated prior to transplantation.

5.5 Diagnostic Testing After Transplant

Once transplantation has occurred, recognition of infection based on clinical symptoms becomes more difficult as the immune suppression used to induce immune tolerance also suppresses the immune systems response to infection. As a result, relying on the presence of fever or localizing symptoms is not adequate for the identification and localization of infection. Furthermore, the list of potential infectious pathogens is significantly larger after transplantation and ranges from common community or hospital-acquired organisms to opportunistic pathogens. Due to the morbidity and mortality associated with many of these infections, early and aggressive diagnostic testing is needed for prompt and accurate diagnosis. Although standard microbiologic testing remains the cornerstone of the diagnostic workup following transplantation, there are several diagnostic challenges and principles that are unique to the immunocompromised host including the role of serologic testing and the use of both direct detection antigen assays and highly sensitive NAT for diagnosing infection and assessing response to treatment.

5.6 Serologic Testing After Transplantation

Although serologic testing is central to the evaluation of the donor and recipient before transplantation, the use of serology for the diagnosis of active infection after SOT and HSCT is of limited value in these populations [43–45]. The attenuation of the immune response as a result of transplantation and the use of immunosuppressive medications make development and detection of antibody responses an unreliable predictor of active infection [43, 45]. For example, studies evaluating serial serology testing for CMV (both IgM and IgG) in SOT recipients failed to predict the development of active CMV disease and did not correlate with CMV viremia [44]. In general, serologic testing should be avoided following transplantation and instead direct detection of infectious pathogens through the use of staining techniques, culture-based methods, histopathology, antigen detection, or molecular methods is recommended.

5.7 Direct Antigen Detection of Infectious Pathogens

Microbiologic staining and culture-based methods as well as histopathology are the most common techniques used for direct pathogen detection in the posttransplant setting and remain the cornerstone of the diagnostic work up in patients with infectious symptoms. Direct antigen detection assays have also been developed to aid in the diagnosis of infectious pathogens in transplant recipients. These tests detect pathogen specific antigens through the use of antigen specific antibody binding and can be performed on various media including serum, bronchoalveolar lavage, CSF, and urine. Various methods are used and can provide either qualitative or quantitative results depending on the platform.

5.8 Nucleic Acid Testing After Transplantation

NAT for the direct detection of pathogens is routinely used in the diagnosis of primary infection or reactivation of latent disease in the posttransplant setting. Depending on the assay used, NAT can be performed directly on blood, urine or other body fluids. Although NAT can be used for the detection of many potential pathogens, it is most commonly employed in the diagnosis of active viral infection.

The use of NAT enhances the ability to detect pathogens with high sensitivity, often before clinical symptoms have developed. This capability allows for a preemptive monitoring approach in which NAT can be performed at regular intervals to identify and potentially treat infections prior to the development of clinical symptoms and disease [7]. This preemptive strategy has been studied for the monitoring of CMV infection following transplantation and is considered a reasonable approach for monitoring the recipient for viral infections including CMV and EBV [7, 46]. In addition to being a reliable tool for the diagnosis of both active and asymptomatic disease, quantitative NAT can also be used to monitor response to treatment and overall treatment duration for certain pathogens [47].

Despite the many advantages of NAT there are several notable disadvantages including high cost and interlaboratory variability. Since many laboratory-developed and commercially available assays were developed prior to the availability of international reference standards, comparison of values between platforms may be difficult. For example, prior to the advent of an international reference standard for CMV developed by the World Health Organization, significant interlaboratory variability had been well documented [7, 48, 49]. As not all nucleic acid assays have reference standards, being aware of the potential variability between laboratories is important in being able to interpret the results effectively.

Another potential challenge with NAT in the posttransplantation setting is differentiating low-level asymptomatic viral replication from clinical disease. Since the most common use of NAT following transplantation is to detect reactivation of latent virus, it is sometimes difficult to determine when a positive nucleic acid test is detecting the presence of low-level background viremia of unclear significance vs. active clinical disease [7, 50, 51]. Unfortunately, established guidelines for cut-off values for NAT have not been established for most infectious pathogens and interpretation is often required based on the clinical scenario. Serial monitoring of NAT, however, can be useful in this setting as significant increases in viral load can indicate the development of clinical disease. For example, for CMV, a greater-than-threefold increase in viral load level over a period of approximately 1 week has been associated with the development of clinical disease requiring treatment [7, 51].

5.9 Diagnostic Testing for Specific Infectious Diseases After Transplant (Table 5-3)

5.9.1 Bacteria

For the majority of bacterial infections, gram stain, culture, and histopathology from the site of presumed infection remain the preferred diagnostic tests. Other viable methods for the detection of bacterial pathogens include direct antigen detection assays and NAT. One example of a direct antigen assay is the urinary *Legionella* antigen test that can be used in addition to culture for the diagnosis of *Legionella* pneumonia [52]. These assays use either ELISA or lateral flow assay (LFA) to detect antigen to *Legionella pneumophila* serogroup 1 which accounts for the majority of infections with *Legionella* [53]. Multiple urinary antigen assays have been FDA-approved for the diagnosis of *Legionella* and a recent meta-analysis revealed a pooled sensitivity and specificity for these tests of 74 and 99 % [54]. A similar urinary antigen test has also been developed for *Streptococcus pneumoniae* detection; however, this test has a more variable sensitivity and specificity as compared to the urinary legionella antigen assays and does not differentiate colonization from active infection, particularly in children [52, 53, 55].

5.9.2 Mycobacteria

The diagnosis of active *Mycobacterium tuberculosis* (MTb) infection relies on microbiologic staining for acid-fast bacilli, culture, and histopathology from the site of presumed infection [56, 57]. Samples can be obtained from many potential sites including sputum (expectorated vs. induced vs. bronchoalveolar lavage), pleural fluid, gastric aspirate, urine, CSF, bone marrow, bone, or blood [56]. If noninvasive staining and culture techniques fail to yield a diagnosis, pursing biopsy for tissue staining, culture, and histopathology is recommended when possible. When pulmonary tuberculosis is suspected and sputum or induced sputum samples are to be used for diagnosis, at least three single specimens preferably collected on three separate days should be submitted so as to increase the sensitivity of detection [56, 58]. When bronchoscopy is pursued, a sputum sample obtained after the procedure is also recommended [56].

NAT has also been developed to aid in the diagnosis of tuberculosis including multiplex assays designed to detect the presence of MTb DNA as well as genetic mutations commonly associated with the development of rifampin resistance [59]. The sensitivity of MTb NAT is significantly enhanced in AFB smear positive individuals [60]. Use of MTb NAT in AFB smear negative respiratory specimens is less sensitive and if performed should be interpreted with caution. Despite the utility of these multiplex assays in the

TABLE 5-3. Laboratory diagnostics by organism

Organism type			Preferred diagnostic procedures
Bacteria	Common typical bacteria		Gram stain
			Culture[a]
			Histopathology
			Direct antigen assay[b]
	Common atypical bacteria	*Legionella pneumophila*	Culture[c]
			Direct urinary antigen assay[d]
		Mycoplasma pneumoniae	Serology[e]
			NAT
		Chlamydophilia pneumoniae	Serology[e]
			NAT
Mycobacteria	Tuberculous		Acid-fast stain[f,g]
			Culture[f,g]
			Histopathology
			NAT[h]
	Nontuberculous		Acid-fast stain[i]
			Culture[i]
			Histopathology
			Nucleic acid probes[j,k]
Fungi	*Candida*		Fungal stain
			Culture[l]
			Histopathology
			β (beta)-D Glucan assay[m–o]
			Magnetic resonance/nanoparticle assay
	Aspergillus		Fungal stain
			Culture[p]
			Histopathology[p]
			β (beta)-D Glucan assay[q]
			Aspergillus Galactomannan assay[r–u]
	Pneumocystis jirovecii		Indirect immunofluorescence[v]
			Histopathology[v]
			β (beta)-D Glucan assay[w]
			NAT[x]
	Cryptococcus		Calcofluor stain[y]
			Culture[y]
			Cryptococcal antigen assay[z,aa]
	Endemic fungi		Fungal stain
			Culture[bb]
			Histopathology[bb]
			Direct antigen assays[cc,dd]
Viruses	CMV		NAT[ee–gg]
			CMV pp65 antigenemia[hh]
	EBV		NAT[ii]
	Respiratory viruses	RSV	Direct fluorescent antigen[jj]
			NAT[kk]
			Rapid antigen assay[ll]
		Influenza	NAT[mm]
			Direct fluorescent antigen
			Rapid antigen assay[nn]
		Other	Direct fluorescent antigen assay
			NAT

(continued)

TABLE 5-3. (continued)

Organism type		Preferred diagnostic procedures
Parasites	*Toxoplasma gondii*	Microbiologic examination[oo]
		Histopathology[oo]
		NAT[pp]
	Trypanosoma cruzi	Microbiologic examination[qq]
		Histopathology
		NAT[rr]
	Plasmodium species	Microbiologic examination[ss]
		Histopathology
		Malaria rapid antigen[tt]
	Babesia microti	Microbiologic examination
		Histopathology
		NAT

[a]Multiple different growth media used depending on the type of bacteria expected.
[b]Urinary antigen test available for *Streptococcus pneumoniae* but sensitivity only 52–78 % in patients without bacteremia [53].
[c]Considered gold standard for diagnosis of *Legionella* pneumonia but sensitivity only 25–75 % [91].
[d]Detects *Legionella pneumophila* serogroup 1 only. Sensitivity and specificity are 74 % and 99 % respectively [92].
[e]Preferred over NAT testing. IgM antibody or convalescent serologies performed 2–3 weeks later can be performed [53].
[f]For suspected pulmonary tuberculosis, at least three expectorated or induced sputum samples obtained are required [54, 56].
[g]If bronchoscopy is performed, a sputum sample after the procedure should be obtained to increase detection rate [54].
[h]Detects MTb DNA and genetic mutations associated with rifampin resistance [58].
[i]For suspected pulmonary tuberculosis, three expectorated or induced sputum samples are recommended [59].
[j]Three currently FDA-approved probes: *Mycobacterium avium complex* (MAC), *Mycobacterium kansasii*, *Mycobacterium gordonae* [59].
[k]Sensitivity of nucleic acid probes range from 85 to 100 % with a specificity of 100 % [59].
[l]Considered gold standard for diagnosis of Candidemia but sensitivity only 50 % [61].
[m]Assay has 75 % sensitivity but lacks specificity for the diagnosis of invasive Candidiasis [62].
[n]False positives reported with hemodialysis, human blood products, certain antibiotics and bacterial infections, surgical gauze [61].
[o]Does not provide information on antifungal susceptibility and should not replace traditional diagnostic methods.
[p]Considered gold standard for diagnosis of invasive aspergillosis.
[q]Sensitivity 77–80 % but lacks specificity. Cannot be used primarily for diagnosis [62, 64, 65, 69, 76].
[r]Variable sensitivity depending on test characteristics, use of antifungals, type of *Aspergillus species*, population tested [60, 64, 67, 68].
[s]False positives reported with *Histoplasma* and *Fusarium species*, piperacillin-tazobactam, amoxicillin-clavulanate, cotton or cardboard, certain blood products, and certain intravenous hydration fluids containing sodium gluconate [60, 66, 69].
[t]Can be used for monitoring of serum galactomannan antigen testing for patients with HSCT [66, 67].
[u]91 % sensitive and 88 % specificity when an EIA cut-off value of ≥1.0 used [72].
[v]Considered gold standard for diagnosis and should be performed on induced sputum or BAL samples [73].
[w]95 % sensitivity and 86 % specificity and can serve as a rule-out test when pretest probability is low [60, 69, 76].
[x]NAT with an 87 % sensitivity and 92 % specificity of [75].
[y]Current gold standard for diagnosis with culture being positive in >90 % of patients with central nervous system involvement [77].
[z]Sensitivity of 97 % and a specificity ranging from 93 to 100 % [67, 78].
[aa]Serum cryptococcal antigen assays with lower and more variable sensitivity depending on the site of infection [67, 69].
[bb]Considered gold standard for diagnosis.
[cc]Urine antigen assays for histoplasmosis and blastomycosis with sensitivities of >90 % [81, 82].
[dd]Significant cross-reactivity has been seen across endemic fungi [60].
[ee]Performed weekly as a preemptive monitoring strategy or to diagnose active infection [7].
[ff]Can be used in monitoring response to treatment and treatment duration [46].
[gg]Tests using the international reference standard by the World Health Organization (WHO) recommended [7, 47].
[hh]Slightly lower sensitivity than NAT. Cannot be used in patients with neutropenia [7, 84, 85].
[ii]Serial monitoring of high-risk HSCT and SOT recipients is recommended [2, 7, 42, 83].
[jj]DFA with 77.8 % sensitivity and 99.6 % specificity with variability in these values depending on technician experience [86].
[kk]Many FDA-approved NAT tests with sensitivities greater than 90 % [87].
[ll]Due to low sensitivity, these assays should not be used to exclude infection in the transplant population [53].
[mm]Preferred diagnostic strategy due to superior sensitivity, specificity, and rapid turn-around time [88].
[nn]Due to low sensitivity, follow up testing with either NAT or viral culture is recommended with negative test results [88].
[oo]Sensitivity and specificity dependent on technician experience [53].
[pp]Often only available in reference laboratories [53].
[qq]Low sensitivity, repeated or serial samples recommended [53].
[rr]Not commercially available, only offered through the Centers for Disease Control (CDC) [89].
[ss]Sensitivity and specificity dependent on technician experience. Three separate samples recommended if clinical suspicion [53].
[tt]A negative rapid malaria antigen should be confirmed by blood smear light microscopy [40, 41].

diagnosis of pulmonary tuberculosis, these tests are generally not approved for use on non-respiratory samples and require extensive in-house laboratory validation before routine clinical use. Furthermore, a MTb nucleic acid test does not preclude the need for culture for formal identification and susceptibility testing [59].

For nontuberculous mycobacteria, microbiologic staining for acid-fast bacilli, culture, and histopathology from the site of presumed infection are also the recommended [61]. As with MTb, for presumed respiratory infection diagnosis, three separate sputum samples obtained from either expectorated or induced sputum is required [61]. Smear and culture can also be obtained from other samples such as blood, abscess cavities, bone marrow, and biopsy tissue. Whenever tissue is obtained, the specimen should also be sent for histopathological examination. Although final identification and susceptibility testing is largely determined based on culture, nucleic acid probes for the nontuberculous mycobacteria *Mycobacterium avium complex* (MAC), *Mycobacterium kansasii*, and *Mycobacterium gordonae*, have been approved by the FDA for the diagnosis of these organisms [61]. The sensitivity of these assays range from 85 to 100 % with a specificity of 100 % [61]. However, unlike MTb NAT that can be performed directly on primary specimen, these probes require growth (amplification) in culture before they can be used [61]. Furthermore, nucleic acid hybridization probes do not provide any information on drug susceptibilities.

5.9.3 Fungi

Microbiologic staining, culture, and histopathology remain the gold standard for diagnosis of invasive fungal infections in immunocompromised patients [62]. Unfortunately, these traditional methods can suffer from a low sensitivity depending on the location and type of fungal infection present, which can lead to significant delays in diagnosis and treatment. Newer diagnostic assays such as direct antigen assays and NAT have been developed for many different types of fungi and are can be helpful in the diagnosis of fungal infection in this population.

5.9.3.1 ***Candida***

For invasive infections with *Candida species*, the gold standard in diagnosis remains microbiologic staining, culture, and histopathology from the location of presumed infection. In cases of Candida blood stream infections (Candidemia) blood cultures should be sent on all patients as commercially available automated blood culture systems will detect growth of these organisms [52, 62]. Unfortunately, blood cultures are only 50 % sensitive for the diagnosis of Candidemia, leading to significant delays in diagnosis and treatment [63].

In an effort to improve the diagnosis of both Candidemia and other forms of invasive candidiasis, other diagnostic methods have been developed. Detection of β (beta)-D Glucan antigen, a cell wall component common to many fungal species including Candida, has been widely adopted as a surrogate marker for fungal infection. Although not specific for *Candida* infections, the β (beta)-D Glucan assay has been studied extensively for the diagnosis of invasive candidiasis. A recent meta-analysis of pooled studies revealed that the β (beta)-D Glucan assay had a 75 % sensitivity for the diagnosis of invasive candidiasis [64]. Although this sensitivity is higher than that of standard blood culture, issues surrounding the β (beta)-D Glucan assay's specificity make it a less attractive diagnostic tool. Furthermore, numerous false positive results have been reported in patients receiving hemodialysis, human blood products, and certain antibiotics as well as in patients with bacterial infections or those undergoing wound care with surgical gauze [63]. Finally, the β (beta)-D Glucan assay does not provide any information on antimicrobial susceptibility. Given these limitations, although a potential adjunct to the diagnosis of invasive candidiasis, the β (beta)-D Glucan assay should not replace the traditional diagnostic methods of microbiologic staining, culture, and histopathology.

5.9.3.2 Aspergillus

For invasive infection with *Aspergillus species*, the gold standard for diagnosis remains identification of the pathogen on histopathological specimens and growth in culture [62]. Unfortunately, the sensitivity of culture for the diagnosis of aspergillus is low and specimens for histopathological examination are often more difficult to obtain, leading to a high number of missed cases and significant delays in diagnosis and treatment [65]. For example, a recent study of bronchoalveolar fluid culture in immunocompromised patients with invasive pulmonary aspergillosis found a sensitivity of only 50 % and unlike *Candida*, the yield of blood culture for *Aspergillus* is very low [65]. To improve detection, many new direct antigen assays have been developed to aid in the diagnosis of invasive aspergillosis. One such assay is the β (beta)-D Glucan assay which has a sensitivity of 77–80 % for the diagnosis of invasive aspergillosis [64–66]. Similar issues with the specificity of this assay exist as for those with *Candida* infections, however, making it an unreliable tool for the diagnosis of invasive infection.

The *Aspergillus* Galactomannan antigen assay has also been developed for the diagnosis of invasive aspergillosis. This assay is a direct enzyme immunoassay (EIA) performed on serum that detects the presence of the cell wall component galactomannan which is specific to *Aspergillus species* [62, 67]. Numerous studies have shown a variable sensitivity of this assay for the diagnosis of invasive *Aspergillus* with

sensitivity ranging from 29 to 100 % depending on the test characteristics, the previous use of antifungals, the type of *Aspergillus species* present, and the population being tested [62, 65, 68, 69]. Specificity is significantly higher although cross-reactivity has been reported with other fungal organisms such as *Histoplasma* and *Fusarium species* [62]. False positive results have also been reported with certain antibiotics such as piperacillin-tazobactam and amoxicillin-clavulanate, contamination of a specimen with cotton or cardboard, certain blood products, and certain intravenous hydration fluids containing sodium gluconate [67, 70]. Serum galactomannan antigen testing is best performed as a preemptive monitoring strategy for patients with hematological malignancies and those who have undergone hematopoietic stem cell transplant as the performance of this test has been best validated in these groups [67, 68]. Studies evaluating the sensitivity of this assay in SOT recipients have not shown a benefit and routine screening using this diagnostic method is not recommended [70, 71]. Serum galactomannan antigen testing can also be used to aid in the diagnosis of active infection in transplant recipients with symptoms suggestive of invasive aspergillosis, however, due to its variable test characteristics, it cannot solely be relied on for definitive diagnosis in this patient population [72].

The *Aspergillus* galactomannan antigen assay can also be performed on BAL with improved sensitivity and specificity for the detection of invasive pulmonary aspergillosis. In patients with hematologic malignancies, the BAL *Aspergillus* galactomannan antigen assay was noted to be 91 % sensitive and 88 % specific when an EIA cut-off value of ≥1.0 was used [73]. Similar results have also been seen in patients with SOT with the BAL *Aspergillus* galactomannan antigen assay having a sensitivity of approximately 82 % [71]. False positive results can occur in the setting of colonization of the airways with both *Aspergillus* and non-*Aspergillus* species such as *Penicillium* and careful interpretation of the results based on the clinical setting is recommended [62].

Finally, many new additional diagnostic tests for the diagnosis of invasive aspergillosis are currently being studied and validated. These include lateral flow devices (LFD's), *Aspergillus* NAT, and a breath test evaluating *Aspergillus* specific volatile organic compounds (VOC's) [62]. Although initial studies appear promising, further research needs to be performed before considering their use in the routine clinical setting.

5.9.3.3 Pneumocystis jiroveci

The gold standard for diagnosis of *Pneumocystis jiroveci* (PCP) remains direct visualization of the organism in respiratory tract secretions or histopathology [74]. The most common staining technique used is the indirect IFA which uses *P. jiroveci* specific monoclonal antibodies [75]. Whenever possible, samples from induced sputum or BAL should be performed to increase the sensitivity of detection [74]. NAT has also been developed and studied for the diagnosis of PCP. A recent study looking at NAT on sputum samples from non-HIV immunocompromised patients indicated an 87 % of sensitivity and a specificity of 92 % [76]. Limitations include false positive results in the setting of colonization of the respiratory tract and careful interpretation of the results based on the clinical setting is always recommended. Finally, the β (beta)-D Glucan assay can also be used in the diagnosis of PCP as the organism produces β (beta)-D Glucan. A meta-analysis revealed that this assay has a 95 % of sensitivity and an 86 % of specificity for the diagnosis of PCP, indicating that the likelihood of infection with a negative test result is extremely low [62, 70, 77]. Given this, it can serve as a good test of exclusion when clinical pretest probability is low.

5.9.3.4 Cryptococcus species

Direct visualization of the organism with fungal stain and culture remains the gold standard for the diagnosis of Cryptococcus. Whenever possible, calcofluor fungal staining should be performed over India ink staining due to issues with the sensitivity and specificity of India Ink staining in the presence of CSF leukocytes [78]. Culture for Cryptococcus is positive in >90 % of patients with central nervous system involvement, however, growth on culture can take up to a week which can lead to delays in diagnosis and treatment [62, 79].

Several antigen assays for *Cryptococcus* are widely used for the rapid diagnosis of invasive cryptococcal infection. These assays can be performed on both serum and CSF and detect the presence of the polysaccharide capsule unique to *Cryptococcus* species [68]. Assay techniques include latex agglutination (LA) and enzyme immunoassay (EIA). CSF cryptococcal antigen testing is more sensitive and specific than that of fungal staining and culture with a sensitivity of 97 % and a specificity ranging from 93 to 100 % [68, 80]. Cryptococcal antigen assays can also be run on serum with a slightly lower and more variable sensitivity depending on the site of infection [68, 70]. False positives are rare but have been reported with other fungal species such as *Trichosporon* and with the bacterial species *Stomatococcus* and *Capnocytophaga* [81, 82]. False positives have also been reported with nonspecific binding of serum proteins (such as rheumatoid factor) and can be avoided with the treatment of serum with pronase. In addition, false positive results have occasionally been reported when samples have contacted certain soaps, disinfectants, and starches [70]. False negative results can occur in patients with high Cryptococcal antigen titers resulting in failure of agglutination between antigen–antibody complexes (prozone effect) [83].

This can be avoided through either the addition of pronase to serum or sample dilution if equivocal results are obtained [83, 84]. Overall, however, the CSF and serum cryptococcal antigen assays remain valuable diagnostic tools for the diagnosis of invasive cryptococcal infection. The β (beta)-D Glucan assay is not considered to be an appropriate test for the diagnosis of invasive cryptococcal infection as this cell wall component is not found in this organism [62].

5.9.3.5 Dimorphic Fungi

For the endemic fungi histoplasmosis, blastomycosis and coccidioidomycosis, diagnosis is made largely based on direct examination of the organism on fungal staining, culture, or histopathology. As with Cryptococcus, growth of these organisms can be slow and can lead to delays in diagnosis and treatment [62]. Direct antigen assays for histoplasmosis and blastomycosis have been developed and have been shown to have sensitivities of >90 % for the detection of disseminated infection [85, 86]. Multiple techniques are used including enzyme immunoassay, immunodiffusion, and complement fixation and can be performed on both serum and urine [62]. Significant cross-reactivity has been seen across the three different endemic fungi, however, limiting their overall usefulness in the clinical setting [62].

5.10 Viruses

For viruses, direct antigen detection methods and NAT are the preferred diagnostic screening strategies for patients in the posttransplant setting. Although serologic methods are commonly used in the pretransplant setting, these markers are unreliable in the setting of immune suppression and should not be used for routine diagnosis [43–45]. Both viral cell culture and histopathology can also be performed to aid in the diagnosis of viral infection although utility is limited by low sensitivity, prolonged turn-around time, and the need for more invasive procedures to obtain appropriate specimens [45]. Viral cell cultures from the urine and stool should not be performed as a positive culture often represents asymptomatic shedding and is not necessarily a marker of active disease [45].

5.10.1 CMV and EBV

For CMV and EBV, diagnosis in the posttransplant setting is most commonly made by direct detection of virus using NAT as these assays exhibit high sensitivity and specificity for the diagnosis of infection [43, 45]. Serial monitoring of high-risk HSCT and SOT recipients is recommended in those not receiving chemoprophylaxis so as to identify and treat infection prior to the development of significant disease [2, 7, 43, 87]. Limitations to NAT for CMV and EBV can be found under "Nucleic Acid Testing" in Sect. 5.8.

CMV infection in transplant recipients can also be reliably diagnosed through the use of CMV pp65 antigenemia. This test utilizes monoclonal antibodies to pp65 which can be found in CMV infected white blood cells and can provide a semiquantitative assessment of infectious burden [88]. Studies comparing antigenemia with CMV NAT have shown a slightly lower sensitivity as compared with NAT [89]. Advantages to this assay include its cost and rapid turn-around time [7, 88]. Limitations include its requirement for the presence of white blood cells preventing its use in neutropenic patients, lack of standardization of results, and significant skill and labor involved in processing and microscopy [7, 88]. It can also only be performed on blood whereas NAT testing can be performed on multiple specimen types.

5.10.2 Respiratory Viruses

For the respiratory viruses both direct viral antigen assays and NAT can be used for the diagnosis of infection. For respiratory syncytial virus (RSV), both direct fluorescent antigen testing (DFA) and NAT are the most commonly used diagnostic modalities in transplant patients. DFA has been shown to have 77.8 % sensitivity and 99.6 % specificity for the diagnosis of RSV with variability in these values depending on microscopist experience [90]. There are currently several RSV nucleic acid tests that have been approved by the FDA for use with sensitivities greater than 90 % [91]. It is important to note, however, that transplant recipients can shed virus in the nasal passages for a significantly longer period of time than immunocompetent hosts, requiring clinicians to depend on clinical pretest probability to differentiate active vs. past infection. Rapid RSV direct antigen assays which are typically performed as point of care tests with results within 30 min are also available but due to low sensitivity, these assays should not solely be used to exclude infection in the transplant population [52].

For influenza, NAT is the preferred diagnostic strategy for the diagnosis of infection due to its superior sensitivity, specificity, and rapid turn-around time [92]. Nasopharyngeal samples are most commonly obtained but deeper samples from the lower respiratory tract should be pursued in cases of severe, lower tract infection, due to increased sensitivity of detection of virus [93]. Limitations of NAT include its ability to identify only certain influenza strains felt to be the most prevalent during a given influenza season, leading to potential false negative results in instances of infection with uncommon strains. Direct fluorescent antigen testing is also available and can be performed when NAT testing is not

available [92]. As with RSV, similar issues with persistent viral shedding can occur in transplant recipients making it difficult to distinguish active from resolved or resolving infection. Although rapid influenza antigen detection assays are available for the diagnosis of influenza, given their reduced sensitivity for the detection of infection, follow up testing with either NAT or viral culture is recommended with negative test results [92].

For other respiratory viruses such as Parainfluenza, Adenovirus, and Human Metapneumovirus, both direct antigen detection assays and NAT can be considered for diagnosis of infections in the transplant population. Determination of which assay to use should be individualized by each institution depending on feasibility and cost.

5.11 New and Emerging Diagnostic Tools

Over the past 50 years there have been many new advances in the field of microbiology diagnostics which have transformed our ability to identify a multitude of organisms including bacteria, mycobacteria, viruses, and fungi. Compared to the standard diagnostic techniques which rely on organism growth and subsequent phenotypic identification, many of these new methods focus on the molecular signatures of pathogens through the recognition of microbial DNA, RNA, or proteins [94]. These new techniques can reduce the time to organism identification from initial specimen collection with increased sensitivity and specificity compared to standard methods, improving a physician's ability to make rapid and accurate clinical decisions for ill patients [94]. Many of these technologies have already been incorporated into routine clinical care and should be considered, if available, in the work up of the transplant patient.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a molecular technique that is now FDA-approved for the routine identification of bacteria and yeasts in clinical microbiology laboratories. This technique identifies bacteria and yeast through the ionization of the unique protein structure of each particular organism and measures the mass to charge ratio of each ionized subcomponent [95]. This measurement creates a unique protein signature specific to each organism that can then be compared against an extensive database of known and validated profiles ("spectra") [94, 95]. Studies have shown that MALDI-TOF MS can reliably provide identification at the genus, species, and subspecies level for a wide variety of organisms [96–100]. Analysis is typically performed as soon as colony growth of an organism has occurred and can reduce time to organism identification by 1–1.5 days compared to standard phenotypic methods [101]. Further advantages include its automation, relative ease of use, and its ability to perform high-throughput analysis [95, 102]. Finally, although the initial monetary investment in MALDI-TOF MS is high and laboratory implementation is time consuming; numerous studies have shown that long-term utilization of this technology is cost-effective compared to standard methods [100, 101].

Limitations of the MALDI-TOF include its reliance on an organism database for identification as organisms not present in the database will often not be identified or identified incorrectly [102]. Furthermore, current commercially available libraries are not approved for the identification of most mycobacteria and fungi, however, with time, this is expected to change and may dramatically shorten the time to identification of these traditionally difficult-to-identify organisms. Another limitation of MALDI-TOF MS is that some closely related bacteria may also be misidentified in certain instances [95]. Although research is ongoing, the MALDI-TOF MS currently does not perform susceptibility testing. Finally, despite the significant reduction in time to organism identification, the MALDI-TOF still requires pure culture of an organism and is not yet able to reliably detect organism directly from primary clinical specimens [102].

Molecular diagnostic techniques have also been developed for organism identification directly from positive blood culture specimens without the need for additional subculture and colony growth. The general concept is that nucleic acid amplification occurs with microbial growth and therefore these assays take advantage of novel detection strategies. One technique which has been developed to detect common bacterial pathogens as well as common antimicrobial resistance genes is a nanoparticle microarray assay which can directly be performed on positive blood cultures [103–105]. Divided into panels, these automated assays have shown to have a >90 % concordance rate compared to standard identification and susceptibility techniques [103, 104]. Furthermore, time to result with these assays is shorter than that of standard methods, resulting in significant reductions in time to organism identification and initiation of appropriate antimicrobial therapy [106]. Despite these promising results, there are some limitations to these assays, particularly with polymicrobial infections. In addition, these assays only test for several common bacterial pathogens and resistance markers, requiring the need for further subculture, identification, and susceptibility testing to identify less common bacterial pathogens. For these reasons, these assays are only approved to provide "preliminary" results and must still be confirmed by standard microbiological identification and susceptibility methods [102].

New diagnostic methods are also available for the detection of pathogens from direct clinical samples without the need for growth in the primary specimen or subculture. As

with the above microarray techniques, these tests rely on the identification of pathogen specific DNA or RNA through multiplexed nucleic acid amplification and detection. Although single organism identification NAT's are available and commonly used, newer platforms have been developed which allow for the detection of multiple pathogens in a single sample [91, 107–109]. Multiple FDA-approved platforms are available for the detection of enteropathogens in stool samples and respiratory viruses in nasopharyngeal samples [91, 107–109]. The main advantages to these assays include their high sensitivity and specificity as well as their ability to be run directly on primary specimen, leading to a result time of only hours from specimen collection [108]. This reduced turn-around time is invaluable to clinicians as it can lead to timely management decisions for ill patients. Limitations of the multiplex assays include the high cost and labor requirements as batch testing of samples is not currently available for all assays.

Finally, a technique using magnetic resonance and nanoparticle NAT technology has recently been FDA-approved for the diagnosis of Candidemia. As with the multiplex assays, this technology has the ability to detect five common *Candida* species directly on whole blood without the need for growth in the primary specimen or subculture [110]. Studies have shown that this assay has a rapid turn-around time and a sensitivity and specificity of 91 and 99.4 % for the detection of Candidemia [110]. Although this assay is highly sensitive, routine blood cultures must still be obtained to exclude other diagnoses. Furthermore, this assay does not directly detect antifungal drug resistance. In some cases, however, susceptibility information can be inferred based on organism identification (example, identification of *C. krusei* infers azole resistance).

5.12 Summary

The diagnosis of infectious diseases in the transplant patient requires an extensive evaluation of both the donor and the recipient prior to transplantation as well as a solid understanding of the risk factors present after the procedure. By understanding these factors, a comprehensive differential diagnosis can be made and the appropriate diagnostic testing and treatment can then be initiated. Prompt diagnosis often requires the use of early and aggressive diagnostic testing from a multitude of different diagnostic modalities including microbiologic staining, culture, histopathology, direct antigen detection, and molecular-based techniques such as NAT. By understanding the utility of each of these different diagnostic strategies in different clinical situations, more rapid and accurate diagnoses can be made leading to improvement in treatment and outcomes in this patient population.

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Part II
Risks and Epidemiology of Infections After Transplantation

6
Risks and Epidemiology of Infections After Hematopoietic Stem Cell Transplantation

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6.1 Introduction

Understanding the epidemiology of infections after allogeneic hematopoietic stem cell transplantation (HCT) is important to implement appropriate preventive strategies as well as to effectively diagnose and treat individual patients.

Several groups of experts and professional organizations publish guidelines that provide specific recommendations for prophylaxis and management of infections after HCT [1–8], including vaccinations [1, 9, 10]. Many of these recommendations are necessarily based on low-quality evidence and rely heavily on expert opinion. Guidelines should not be followed blindly, but understood as tools that may help to provide the best possible care.

Risk factors for infection include individual characteristics (e.g., indication for HCT, prior infections, CMV serostatus, particular genetic traits) and type of transplant (based on conditioning regimen, stem cell source, degree of HLA homology, and immunosuppression). The development of graft-versus-host disease (GVHD) is frequently the decisive contributor to infectious morbidity and mortality.

6.2 Individual Characteristics and the Risk of Infection

Different indications for HCT are associated with their own infectious risks. Primary immunodeficiencies (PID), hemoglobinopathies, and hematologic malignancies present different challenges. Even in hematologic malignancies, the risk may vary depending on the specific condition: patients with chronic myelogenous leukemia (CML), acute myeloid leukemia (AML), and chronic lymphocytic leukemia (CLL) present different risks based on both the biology of the disease and prior treatment. These factors should be considered when assessing individual patients.

Prior infections must be considered. A history of infection or colonization with a multidrug-resistant organism (MDRO) like carbapenem-resistant enterobacteria (CRE), extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacteria, vancomycin-resistant enterococcus (VRE), or methicillin-resistant *Staphylococcus aureus* (MRSA) has implications regarding optimal management of fever during neutropenia [6, 11, 12], which is a common complication of HCT. Transplant candidates are routinely screened for serologic evidence of latent infections that may reactivate (HSV, VZV, CMV, EBV, hepatitis B and C, toxoplasmosis); some of these will be discussed later in this chapter. Some transplant centers will perform screening for tuberculosis with tuberculin skin test (TST) or interferon-gamma release assay (IGRA), at least for patients who are considered at significant risk for the disease. Prior invasive fungal infections may reactivate following transplant, and secondary prophylaxis is required [13–15]. Even active fungal infection has been reported to be controllable. There are, however, cases of progression of prior aspergillosis after transplant; myeloablative conditioning, prolonged neutropenia, cytomegalovirus (CMV) disease, and graft-versus-host disease (GVHD) are risk factors [15, 16].

As the correlates of native and adaptive immunity are better understood, genetic associations are coming to light. There is evidence that some donor haplotypes of *TLR4*, the gene that encodes the toll-like receptor protein 4 (TLR4) are associated with increased risk of invasive aspergillosis after HCT [17]. Recipient's mutations in *MBL2*, the gene that encodes mannose-binding lectin (MBL), have been associated with increased risk of infection after neutrophil recovery following myeloablative transplant [18]. Other polymorphisms of *MBL2* may be important for infection through a direct influence on the risk of developing GVHD [19, 20]. Different genotypes of activated killer immunoglobulin-like receptors (aKIR) in the donor have been found to protect from CMV reactivation [21]. Many of these associations are preliminary and require more data to be confirmed, but they hold the promise of a more individualized approach to infectious prophylaxis.

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6.3 Time Course of Infections After Allogeneic Stem Cell Transplantation

From a practical standpoint, it is helpful to consider three distinct periods during transplant: pre-engraftment (until neutrophil recovery), early post-engraftment (from engraftment until day 100), and late post-engraftment (after day 100). This framework originated with myeloablative transplants, and is eminently pragmatic. The pre-engraftment phase may be accompanied by profound neutropenia and significant mucositis, which results in increased risk of bacterial infections from the resident gastrointestinal flora, candidiasis, aspergillosis (in cases of prolonged neutropenia) and herpes simplex virus reactivation. After engraftment, with neutropenia no longer being a factor, many infections are related to the profound defect in cellular immunity caused by the conditioning regimen and the immunosuppression administered to prevent GVHD. CMV reactivation and the development of acute GVHD and its treatment play a central role during this time. The day 100 landmark derives from the standard time at which immunosuppression (e.g., cyclosporine A or tacrolimus) is frequently tapered. Infections after this point would be primarily related to lack of immune reconstitution and, in the absence of GVHD, become progressively less common.

6.4 Types of Allogeneic Hematopoietic Stem Cell Transplantation (HCT)

Not all allogeneic stem cell transplantations are the same. Several characteristics of the transplant influence the risk of infection: the conditioning preparative regimen, the source of stem cells, the degree of HLA identity between donor and recipient, and the prophylactic strategy adopted to prevent GVHD (use of T cell depletion or immunosuppressive medications). Table 6-1 summarizes the impact of these factors on infections.

TABLE 6-1. Type of transplant and infectious disease risk

Factor	Type of transplant	Risk of infection
Conditioning regimen	Myeloablative	In general, there are less early infections (mainly bacterial) with nonmyeloablative transplants, but different regimens may have very different risks
	Reduced intensity Nonmyeloablative	Nonmyeloablative regimens do not seem to result in less late infections
HLA match	HLA-matched sibling	With higher degree of mismatch, more immunosuppression is required, immune reconstitution is delayed, and the risk of infection is higher. Haploidentical and partially matched transplants often incorporate T cell depletion
	HLA-matched unrelated (URD or MUD)	
	Haploidentical	Haploidentical transplants using posttransplant cyclophosphamide seem to have good immune reconstitution
	Partially matched	
Source of stem cells	Bone marrow	G-CSF-mobilized peripheral blood stem cells often result in shorter neutropenia, but may be associated with higher risk of chronic GVHD. Conflicting data on CMV risk
	G-CSF-mobilized peripheral blood stem cells	UCD transplants result in long-lasting neutropenia and prolonged immunodeficiency, with higher risk of infection
	Cord blood (UCD)	High risk of viral infections with cord transplants
GVHD prophylaxis (posttransplant immunosuppression)	T cell depletion (in vitro via CD34+ cell selection or in vivo with ATG or alemtuzumab)	T cell depletion results in increased risk for infections. ATG and alemtuzumab may result in prolonged lymphopenia and immunodeficiency, depending on the dose used. Viral infections, EBV-related PTLD, and toxoplasmosis seem to be more common after T cell depletion
	Immunosuppressive agents	Differences between pharmacological immunosuppressive regimens are not well defined; sirolimus may be associated with less CMV reactivation

G-CSF granulocyte-colony-stimulating factor, *GVHD* graft-versus-host disease, *CMV* cytomegalovirus, *ATG* anti-thymocyte immunoglobulin, *EBV-related PTLD* Epstein–Barr virus-related posttransplant lymphoproliferative disorder.

6.4.1 Preparative (Conditioning) Regimen

The conditioning regimen administered before the infusion of stem cells has some influence on the risk of infection through its effect on neutropenia, mucosal damage, and GVHD. The conditioning regimen has several goals: reduction of the malignancy (when there is one), creation of space in the bone marrow to provide a selective advantage to the infused stem cells, and elimination of the recipient's immune system to minimize the risk of rejection. Different conditioning regimens may be more appropriate depending on the disease and the general status of the recipient [22]. Myeloablative, reduced intensity, and nonmyeloablative are the general categories, but within each one there are substantial differences that may be relevant. In general, fully myeloablative regimens result in more prolonged neutropenia and more severe mucosal barrier damage, which may impact the infectious risk during the pre-engraftment period [23].

6.4.2 Degree of HLA Similarity Between Donor and Recipient

Data from the Center for International Blood and Marrow Transplant Research (CIBMTR) indicate that there is a direct association between the number of donor–recipient HLA mismatches and the risk for mortality [24]. The current standard aims for high-resolution matching at HLA-A, HLA-B, HLA-C, and HLA-DRB1 (i.e., an "8 out of 8" match), but only about 30% of transplant candidates will have a perfectly matched sibling or unrelated donor (MUD). If a mismatch is unavoidable, a single-locus mismatched donor can be used [24]. Other alternatives include haploidentical and umbilical cord blood (UCB) transplants.

Haploidentical transplants are one special type of mismatched transplant, where the donor shares at least one complete haplotype with the recipient. Most candidates for transplant have a potential haploidentical donor. The successful use of a regimen of posttransplant cyclophosphamide to prevent GVHD in the haploidentical setting has resulted in an increasing number of this type of transplant being performed during the last decade [25]. Interestingly, early data suggest haploidentical transplants do not result in delayed immune reconstitution or increased infections [26].

Matching for UCB transplants focuses on three loci (HLA-A, HLA-B, and HLA-DRB1). The majority of UCB transplants are mismatched by at least one locus (often two). Among transplants mismatched at two loci, mismatching at HLA-C and HLA-DRB1 was associated with the highest risk of mortality [24].

The degree of mismatch between the donor and the recipient affects the infectious risk mainly through the likelihood of GVHD. More GVHD usually results in more infections. To prevent GVHD in a mismatched transplant, more potent immunosuppression may be required, increasing the risk of infection. It is also possible that immune reconstitution proceeds more slowly (even with the same immunosuppressive regimen) after a URD HCT. These factors may result in increased risk of infections associated with T cell immunodeficiency, like CMV, *Pneumocystis jirovecii* pneumonia (PCP), and Epstein–Barr virus (EBV)-related posttransplant lymphoproliferative disorder (PTLD).

However, provided the number of stem cells administered is the usual ($>3\times10^6$ kg^{-1}), neutrophil recovery proceeds at the standard pace and there is no increased risk of neutropenia-related infections.

The problems with UCB transplants include a markedly decreased stem cell dose (often $<1\times10^5$ kg^{-1}) which results in prolonged neutropenia (up to 6 weeks), with the attendant risk of bacterial and fungal infections [27]. In addition, the cord blood does not have antigen-specific memory T cells that can expand in a thymus-independent fashion to provide protection against viruses and opportunistic pathogens. This results in high frequency of late severe infections following cord transplantation, even when the neutropenic period is shortened by coadministration of stem cells from a third-party donor [28].

6.4.3 Source of Stem Cells

Stem cells may be given using the bone marrow, G-CSF-mobilized peripheral blood stem cells (PBSCs), or UCB. Frequently bone marrow will result in more prolonged neutropenia compared with PBSC, and increased infections during neutropenia should be expected. However, a multicenter randomized trial comparing peripheral blood stem cells with the bone marrow from unrelated donors showed no difference in the relapse or infectious mortality between both groups, but confirmed that chronic GVHD is more common with mobilized PBSC [29]. The particular features of UCD transplants were discussed on the preceding paragraph.

6.4.4 Strategy to Prevent GVHD: Manipulation of the Stem Cells, Immunosuppressive Drugs, or a Combination

GVHD may be prevented by decreasing the amount donor T cells or by limiting T cell function with immunosuppressive agents. The stem cells, whether from the bone marrow or the periphery, may be administered unmanipulated (sometimes called "T cell replete") or enriched by CD34 selection (also called "T cell depleted"). If unmanipulated bone marrow or PBSCs are used, the dose of CD3+ T cells administered with the graft varies between 24×10^6 kg^{-1} when bone marrow is used and 300×10^6 kg^{-1} when PBSCs are used [30]. Reductions in the amount of T cells of 2–3 $\log_{10}$ are possible,

and in some haploidentical transplant regimens, as few as 12.5×10^3 CD3+ cells are given, which still results in detectable immune reconstitution starting 2–3 months after transplant [31]. T cell depletion may minimize or altogether prevent GVHD but may result in prolonged immunodeficiency, depending on the degree of depletion. If an unmanipulated product is used, T cell depletion may be attained in vivo by using alemtuzumab or ATG. These agents produce a profound depletion of T cells in vivo, and their long half-life makes them still be present and active in the recipient when the stem cell product is administered.

If no in vitro or in vivo T cell depletion is used, one of a variety of immunosuppressive regimens will be given to prevent GVHD (e.g., tacrolimus + methotrexate, tacrolimus plus mycophenolate mofetil, cyclosporine A, sirolimus, posttransplant cyclophosphamide). A randomized controlled trial documented more infections in patients randomized to (moderate) T cell depletion than in the group who received pharmacologic immunosuppression [32]. T cell depletion in vivo with alemtuzumab has been associated with increased risk of infection [33]. It is possible that different pharmacological regimens may result in different infectious risks, but this has not been adequately studied. Preliminary evidence suggests that a sirolimus-based regimen may result in less CMV reactivation [34] and that posttransplant cyclophosphamide result in relatively decreased risk of PTLD [35].

The above categories may combine in several ways, compounding the risk of infection. These variations should be considered both when designing a regimen of anti-infective prophylaxis and when considering an individual patient who may have an infection.

6.5 Graft-Versus-Host Disease

GVHD is the most important cause of non-relapse mortality following HCT, and it is frequently complicated by infection. GVHD is categorized as acute or chronic based on its time of onset. Acute GVHD develops before day 100 and is characterized by gastrointestinal disease (secretory diarrhea, nausea, vomiting), liver dysfunction, and skin rash. Stages of GVHD in the skin, gut, and liver combine to give a grade (I–IV) of the severity of the disease. Acute GVHD grades III–IV is associated with significant mortality. The treatment of choice is high-dose systemic corticosteroids. GVHD is associated with significant immune dysregulation [36, 37] and is frequently accompanied by CMV reactivation [38]. The combination of disruption of the GI mucosa (and sometimes skin) and high-dose corticosteroids (in addition to the immunosuppressive agents concurrently given, like tacrolimus and MMF) constitute a high-risk setting for infection. Bacterial, fungal, and viral infections are common under these circumstances.

Chronic graft-versus-host disease (cGVHD) has been traditionally defined chronologically: GVHD starting after day 100. It has been classified based on its relation to prior GVHD (progressive when acute GVHD continues after day 100, quiescent when there is a period of time during which the patient is free of GVHD, or de novo when chronic GVHD is the first manifestation of GVHD) and its extension (limited or extensive, reformulated as clinical limited, or clinical extensive). The clinical syndrome of typical chronic GVHD is quite distinct from the acute form, and a new classification focusing on the clinical characteristics of the disease as well as on the timing is being increasingly used [39]. From the standpoint of infectious diseases, the important consideration is that the presence of chronic GVHD is associated with high risk of infection [40, 41]. Multiple immune defects have been described during chronic GVHD, involving humoral and cellular immunity [42, 43] as well as functional hyposplenism [44, 45]. Besides these abnormalities, that result in delayed immune reconstitution and poor response to immunizations, the risk is of infection is increased by the treatment of extensive cGVHD [41], which typically includes systemic corticosteroids and a variety of steroid-sparing agents. Notably, cGVHD is a well-documented risk for pneumococcal infections [45, 46], fungal infections, and late CMV disease. However, all types of infections are more common during cGVHD, particularly during the first few months [47].

When GVHD is not controlled by corticosteroids, it is called "steroid refractory," and there is currently no universally accepted standard treatment. This situation is important from the infectious disease standpoint because patients are usually treated with a variety of highly immunosuppressive regimens (e.g., ATG, cyclophosphamide, MMF, infliximab, daclizumab, alefacept, alemtuzumab, sirolimus, visilizumab, denileukin diftitox, and others) [48] that result in a wide array of infectious complications. Reactivation of CMV is very common, as are fungal infections [49, 50], Epstein–Barr virus-related PTLD [51], as well as human herpesvirus 6 (HHV-6) [52] and adenovirus [53]. There are no controlled studies to support any particular infection prevention strategy during this period of increased immunosuppression, but some authors have emphasized that early use of prophylactic antibiotics and antifungals is an essential part of a successful approach to this problem [54]. Unfortunately, this is a condition for which controlled trials are unlikely to be performed, and different centers will have to decide on a particular approach of close monitoring versus prophylaxis based on local experience and published case series.

In the following sections, the epidemiology of bacterial, fungal, viral, and parasitic diseases will be discussed. The implications for prophylaxis and management will be mentioned. Immunizations for transplant recipients, (as well as their caregivers and immediate contacts) are discussed in Chap. 48

6.6 Risks and Epidemiology of Bacterial Infections After Allogeneic HCT

6.6.1 Early Bacterial Infections: Pre-engraftment

Approximately 20% of HCT recipients will experience at least one episode of bacteremia during the first few weeks, and a similar proportion after engraftment [55]. These infections are usually related to either neutropenia with subsequent bacterial translocation through the GI mucosa (mucosal barrier injury laboratory-confirmed bloodstream infection or MBI-LCBI) or the intravascular catheter (central line-associated bloodstream infections or CLABSIs) [56].

The relative frequency of Gram-positive and Gram-negative infections during neutropenia varies in different series and with the use of prophylactic antibiotics. In some centers, the most frequent Gram-positive isolates are *viridans* group *Streptococcus* [55]; this may be a function of the conditioning regimen or the patient population. *Enterococcus faecium*, frequently VRE, is another Gram-positive organism that tends to cause bloodstream infection relatively early, although this seems to be rather institution dependent [57]. The Gram-negative bacteria are commonly *Enterobacteriaceae*. These infections are generally related to the disruption of the GI mucosa due to the preparative regimen. The role of reduced diversity of the microbiota with subsequent bacterial domination and ultimately bacteremia is an area of intense study [58]. The risk of bacteremia during neutropenia may be decreased by the use of prophylactic antibiotics [59, 60]. This had been shown in multiple studies over the years, but the recommendation of using antibiotics did not become part of practice guidelines until recently. It is not clear whether this recommendation will continue amidst the increasing concern over the role of antibiotic-induced decreased microbiome diversity on the outcome of HCT [61]. In this regard it is of interest that fluoroquinolones seem to have less detrimental effects on biodiversity of the fecal flora than beta-lactams. Levofloxacin at a dose of 500 mg/d for patients who are going to be profoundly neutropenic for longer than 1 week is the current recommendation of the IDSA [11].

6.6.2 Early Bacterial Infections Following Engraftment

In a large study from the Sloan Kettering Cancer Center, the risk factors for post-engraftment bacteremia included acute GVHD, renal dysfunction, hepatic dysfunction, and neutropenia [55]. *Enterococcus* (VRE) and coagulase-negative *Staphylococcus* were the most common Gram-positive isolates. *Enterobacteriaceae* and non-fermentative Gram-negative bacteria (including *Pseudomonas*, *Stenotrophomonas*, and *Acinetobacter*, possibly related to the indwelling catheter) were the most common Gram-negative isolates. Bacteremia following engraftment often happens in the setting of patients with a complicated clinical course, acute GVHD, and multiple medical problems or else is catheter related.

Daily bathing with chlorhexidine-impregnated washcloths decreased the risk of acquisition of MDROs and development of hospital-acquired bloodstream infections in transplant recipients in a randomized trial [62], and this practice should be considered by every transplant program.

The advantages and disadvantages of active screening for colonization by resistant pathogens have not been adequately studied in HCT recipients. It is likely that local epidemiology determines whether screening is an efficacious and cost-effective approach to either prevent infection or improve outcomes. A retrospective study on VRE bacteremia from the Sloan Kettering Cancer Center showed that VRE carriage was predictive of subsequent VRE bacteremia, but failed to detect the pathogen in many patients [63]. Performing surveillance cultures for resistant organisms in vulnerable patient populations is part of the CDC recommendations "Management of Multidrug-Resistant Organisms in Healthcare Settings, 2006" [64], and has been vigorously advocated by some experts [65].

6.6.3 Late Infections: *Streptococcus pneumoniae* and Others

HCT recipients are at high risk for *Streptococcus pneumoniae* infections (2–8.6/1000 patients transplanted) [66, 67]. Both early and late (beyond day 100) pneumococcal disease has been reported, with late infections strongly associated with active cGVHD [46]. These have been attributed to inadequate antibody production and functional hyposplenism [44, 67]. Vaccination against *S. pneumoniae* should be given to all HCT recipients, starting 3–6 months after transplant and using the 13-valent conjugate vaccine [9] (see Chap. 48 for details). Four doses of the vaccine result in enhanced antibody response and tolerable side effects [68]. Antibiotic prophylaxis against *S. pneumoniae* prophylaxis for adults with active cGVHD has been recommended [69], although there is only weak evidence supporting its efficacy. Penicillin V-K is safe and well tolerated, but the local patterns of penicillin resistance may make other antibiotics (e.g., trimethoprim, sulfamethoxazole, azithromycin, or levofloxacin) preferable, although their long-term safety is not well established.

Late bacterial infections often involve the respiratory tract. Pneumonia is the most common cause of fatal late infection [40, 70]. Chronic GVHD is the risk factor most commonly identified. Besides *S. pneumoniae*, multiple other pathogens have been reported. Nocardia also tends to occur

late and in patients with cGVHD [71, 72]. Mycobacterial infections are uncommon and difficult to diagnose [73]. Risk factors for the development of active TB include GVHD, corticosteroid treatment, and total body irradiation (TBI) [74]. The need for universal testing for tuberculosis is controversial, given the unknown sensitivity and specificity of the tests in this population and the fact that tuberculosis is a relatively uncommon complication after HCT (albeit still approximately three times higher than in the general population) [74].

6.7 Risks and Epidemiology of Fungal Infections After Allogeneic HCT

It is necessary to separate invasive candidiasis and candidemia (often related to neutropenia or to the intravenous catheter) from invasive mold infection (of which invasive aspergillosis (IA) is by far the most frequent) [75] (Table 6-2). When deciding on a prophylaxis strategy, it is recommended to consider what kind of fungal infection one is trying to prevent.

Invasive candidiasis follows prior colonization and favorable conditions for the yeast: disruption of the GI mucosa during chemotherapy or acute GVHD, overgrowth in the presence of broad-spectrum antibiotics, and/or presence of indwelling catheters (the catheter seems to be the main risk factor in the case of *C. parapsilosis*). Early studies showed that fluconazole during the pre-engraftment period could decrease the incidence of invasive candidiasis [76, 77]. Accordingly, fluconazole is recommended as part of the standard prophylactic regimen during the pre-engraftment period. The prevalent use of fluconazole has resulted in substantial decrease in the incidence of infections caused by *C. albicans* with relative increases in the incidence of other species of Candida with decreased susceptibility to this agent (e.g., *C. glabrata*, *C. krusei*) [78].

Invasive aspergillosis occurs during specific "at risk" periods following HCT, with a first peak around the time of neutropenia pre-engraftment, a second peak between days 40 and 70 (the time of acute GVHD and its treatment), and a third peak late after transplant, usually in the midst of actively treated cGVHD [79] (Figure 6-1). A variety of risk factors for invasive aspergillosis have been identified over the years, but the most consistently found to be significant in multivariate analyses are acute GVHD, chronic extensive GVHD, and CMV disease [80–82]. Systemic corticosteroids are almost always present as part of the treatment of acute and chronic GVHD.

Non-aspergillus mold infections (e.g., fusariosis, mucormycosis, scedosporiosis), sometimes referred to as emerging mold infections, have been reported with increasing frequency [83]. The increased use of prophylaxis with activity against Aspergillus would be expected to result in a relative increase of other opportunistic mycoses like mucormycosis [84].

Considering the diversity of fungal infections after transplant and the current antifungal armamentarium, it is controversial which antifungal prophylaxis is appropriate at what point during transplant. For instance, although fluconazole is a safe and well-established intervention during the pre-engraftment period of myeloablative transplants [76, 77], it is reasonable to question how necessary it is in transplants with conditioning regimens that result in shorter neutropenia.

Table 6-2. Risk factors and epidemiology of fungal infections after HCT

Pathogen	Risk factors	Comment
Candida spp.	Neutropenia, mucositis, indwelling catheter, heavy colonization, TBI	Non-*albicans Candida* is increasing; *Candida albicans* breakthrough is usually associated with fluconazole resistance
Aspergillus spp.	Prolonged neutropenia	Aspergillus is the most common mold infection in a proportion 7:1 to 9:1 in most series. Antifungal prophylaxis with voriconazole or echinocandins increases the likelihood of non-aspergillus molds
	Type of transplant: cord blood, T cell depletion, partially matched transplant	Not all species of *Aspergillus* are equally invasive or equally susceptible to antifungal agents
	GVHD, acute GVHD and chronic extensive GVHD; systemic corticosteroids	
	CMV disease	
Other molds		
Mucormycosis (formerly zygomycosis)	Prophylaxis with voriconazole	Simultaneous disease of sinuses and the lung was identified as suggestive of mucormycosis in a case–control study
Fusarium spp.	HLA-mismatched transplant	Paronychia and positive blood cultures common
	Prolonged neutropenia	
	Smoking	
Scedosporium spp.	Neutropenia, GVHD, environmental exposure, voriconazole	*Scedosporium prolificans* more invasive and refractory to treatment than *S. apiospermum*

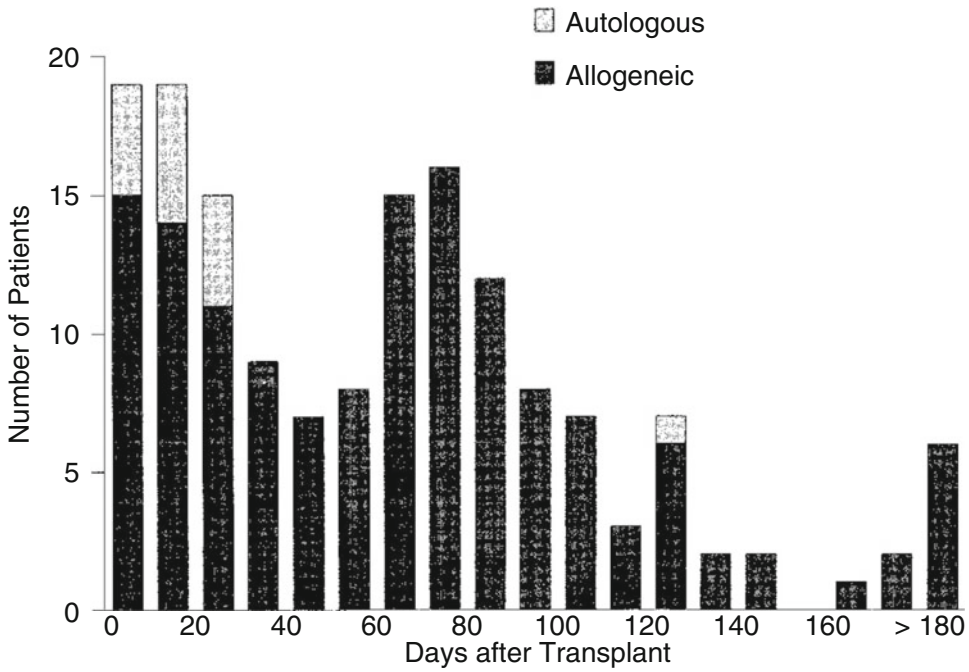

FIGURE 6-1. Time from transplant to diagnosis of aspergillosis in days (From Wald A, Leisenring W, van Burik JA, Bowden RA. Epidemiology of aspergillus infections in a large cohort of patients undergoing bone marrow transplantation. J Infect Dis 1997, Jun;175(6):1459–66, with permission).

Micafungin showed to be equivalent to fluconazole in a randomized controlled trial [85], and the same question (what kind of transplant patient would benefit most) applies.

Regarding the duration of antifungal prophylaxis, fluconazole up to day 75 posttransplant was associated with improved survival mainly due to decreased incidence of systemic candidiasis [86], but it is uncertain whether this strategy should be used for all patients or should be received for some selected subgroups considered at higher risk. Similarly, it is reasonable to question the indication for fluconazole during periods when the main fungal infection is aspergillosis. Several randomized controlled trials have compared fluconazole with another azole with activity against molds (itraconazole [87, 88], voriconazole [89], or posaconazole [90]) either as standard posttransplant prophylaxis or during periods of increased risk. The general conclusion of these trials is that the aspergillus-active drugs are, indeed, more effective than fluconazole in preventing IA, but the benefit in survival in the context of a clinical trial with careful monitoring of galactomannan antigen is hard to demonstrate [91]. The 2009 ASBMT/EBMT Guidelines recommend posaconazole or voriconazole as antifungal prophylaxis in the setting of GVHD and micafungin in the setting of prolonged neutropenia [1]. Of note, posaconazole prophylaxis was superior to fluconazole or itraconazole and improved survival in prolonged neutropenia in non-transplant patients [92]. Now, there are even more options of mold-active prophylaxis with posaconazole delayed-release tablets, intravenous posaconazole, and the new agent isavuconazole.

6.8 Risks and Epidemiology of Viral Infections After Allogeneic HCT

Viral infections remain a challenge because newer transplant modalities result in severe prolonged T cell immunodeficiency and because the current antiviral armamentarium is very limited. Multiple latent viruses may reactivate following HCT [93]. The role of monitoring by PCR is well defined mainly for CMV. Latent viral reactivation is of particular concern in recipients of cord [94] or T cell-depleted transplants. Table 6-3 presents a summary of this section.

6.8.1 Herpesviruses

Members of the herpesvirus family that have caused significant disease after transplant include HSV-1, HSV-2, VZV, EBV, CMV, and HHV-6. Posttransplant complications of HHV-7 are not well defined, although multiple associations have been described. HHV-8 infection and disease (primary effusion lymphoma and Kaposi's sarcoma) occur only infrequently after HCT.

6.8.1.1 Herpes Simplex Virus

HSV-1 and HSV-2 may reactivate following the preparative regimen and complicate chemotherapy-induced mucositis, so it is customary to administer prophylaxis with acyclovir or valacyclovir at least until engraftment. In patients with common recurrences, long-term suppression may be appropriate.

TABLE 6-3. Risk factors and epidemiology of viral infections after HCT

Pathogen	Risk factors	Comment
Respiratory virus		
Respiratory syncytial virus (RSV)	Pre-engraftment	Progression to pneumonia is associated with older age and lymphopenia
	Lymphopenia	It may be less common in nonmyeloablative or reduced intensity transplants
	Preexisting obstructive airway disease	
Parainfluenza	Unrelated donor (URD) transplant	Progression to pneumonia (less common than in RSV) is associated with corticosteroid use and lymphopenia
	CD4+ lymphopenia	
Influenza	Advanced disease	Progression to pneumonia seems less in patients who are receiving corticosteroids
	Female sex	
	Transplantation during influenza season	
Adenovirus	Lymphopenia (T cell depletion), anti-T cell antibodies, umbilical cord blood transplants, mismatched transplants (other than DRB1), haploidentical transplants	Both reactivation of latent adenovirus and new infections occur. Plasma viremia is an important predictor of disease
	Refractory GVHD	
	GVHD on corticosteroids	
Others (metapneumovirus rhinovirus, coronavirus, enterovirus, bocavirus)	Risk factors not well defined	
Herpesvirus		
HSV	HSV + serology in the recipient	
Acyclovir-resistant HSV	Low-dose prophylaxis	
	Intermittent treatment	
	HSV-seronegative donors	
Varicella zoster virus (VZV)	VZV + serology	Clinical reactivation of 25% in the first year after stopping acyclovir prophylaxis
		HCT recipients with multidermatomal zoster should be on airborne and contact precautions
CMV (early disease)	CMV + serology in recipient	Rate of CMV infection in seronegative recipients of seropositive donor (R–/D+) is very low if leucodepleted products are used
	URD transplants and mismatched transplants (in some studies)	
	T cell depletion {Holmberg, 1999 #131}	
CMV (late disease)	Chronic GVHD	
	Corticosteroids	
	CD4+ lymphopenia (<50)	
	Unrelated transplants	
	Haploidentical transplants	
	Umbilical cord blood transplants	
	T cell-depleted transplants	
Epstein–Barr virus (EBV)-related posttransplant lymphoproliferative disorder (PTLD)	Profound T cell cytopenia	
	T cell depletion	
	Anti-T cell antibodies	
	UCB transplants	
	Haploidentical transplants	
Human herpesvirus 6 (HHV-6)	UCB	Reactivation after transplant is very common; disease is rare; multiple disease associations described
	Unrelated donor transplant	
	Mismatched transplant	
	GVHD	
BK virus	Reactivation almost universal after allo-HCT	High-level viremia associated with disease

6.8.1.2 Varicella Zoster Virus

VZV predictably reactivates following transplant (approximately 25% in the first year), either as shingles, multidermatomal, disseminated, or even without a rash ("zoster sine herpete"). In patients who are at risk for VZV reactivation, the use of long-term acyclovir safely prevents the occurrence of VZV disease [95, 96], and currently it is recommended for at least 1 year following HCT.

6.8.1.3 Cytomegalovirus (CMV)

CMV remains latent in a variety of human cells. CMV-seropositive HCT recipients are at risk for CMV reactivation and disease after transplant. The term "CMV infection" is used to denote the presence of CMV in the blood detected by PCR or pp65 antigenemia [97]. Following reactivation, CMV may cause disease typically in the form of pneumonia and/or gastrointestinal disease (most commonly colitis). Other CMV diseases like retinitis or CNS involvement are rare after HCT but have been described: retinitis has been associated with high CMV viral load [98] sometimes in the context of chronic GVHD and CNS disease (encephalitis and ventriculitis), sometimes with resistant virus in the CNS [99, 100].

The risk for reactivation may be related to the presence of CMV-specific immunity in the donor. The rate of CMV infection in the donor–recipient (D/R) pairs often follows the progression $D-/R+ > D+/R+ \gg D+/R- > D-/R-$, suggesting that CMV-specific memory T cells administered with the stem cells may play a role in preventing reactivation and disease. CMV infection or disease in CMV-seronegative recipients of seronegative donors (R−/D−) is rare when leucodepleted or CMV-negative blood products are used [101].

Every transplant program must decide on a strategy to monitor CMV and prevent disease. Depending on a variety of factors, either universal prophylaxis with ganciclovir up to day 100 or a preemptive strategy of weekly monitoring and early therapy may be used. Both approaches resulted in similar overall mortality when compared in a randomized controlled trial, but universal prophylaxis was followed by more cases of late CMV disease [97, 102]. Late CMV disease has emerged as a significant problem, as it occurs when patients are not being under close monitoring by the transplant center. Risk factors include lymphopenia and chronic GVHD [103]. Preventing late CMV disease may be accomplished by either prophylaxis with valganciclovir or the preemptive approach with weekly CMV PCR monitoring [104]. The effect of CMV serostatus of donor and recipient on overall survival is complex (for a review, see [105] and Chap. 24).

6.8.1.4 Epstein–Barr Virus and Posttransplant Lymphoproliferative Disorder

PTLD is a spectrum of lymphoid proliferations that may happen after solid organ or allogeneic stem cell transplantation, usually (but not always) driven by EBV [106]. Pathologically the spectrum goes from polymorphic, polyclonal tissue infiltration of lymphocytes to monomorphic involvement with high-grade B cell lymphoma. After allogeneic HCT, the proliferating cells may be from donor (most commonly) or recipient origin. This disorder is typically related to insufficient or abnormal T cell responses against EBV [107], and accordingly it is more common in the setting of HLA-mismatched transplants, T cell depletion, or intense immunosuppression for the treatment of GVHD [108–110]. Some cases have followed the use of alemtuzumab for in vivo T cell depletion or GVHD prophylaxis [110], despite the fact that anti-CD52 also results in depletion of B cells and earlier had been reported to be associated with relatively less risk. Interestingly, the use of posttransplant cyclophosphamide to prevent GVHD seems to be associated with lower risk of PTLD [35]. Monitoring of EBV viral load by quantitative PCR is now recommended in those transplants considered at high risk. Preemptive management of increasing EBV viral load in patients at risk has been associated with good outcomes [111], although it is not clear when exactly this treatment should be given. A CT/PET may be useful to localize areas amenable to biopsy (Figure 6-2).

6.8.1.5 Human Herpesvirus 6

HHV-6 is acquired early in life, when it may cause roseola infantum and nonspecific febrile illnesses. It frequently reactivates following HCT. Using quantitative PCR, HHV-6 can often be detected in peripheral blood 2–5 weeks after transplant. Most of the time the reactivation seems to be asymptomatic [112], but a number of associations (rash, delayed engraftment, GVHD, thrombocytopenia, increased overall mortality) as well as actual clinicopathological entities (hepatitis, pneumonitis, encephalitis) have been described [113–115]. HHV-6 is possibly the most common cause of infectious encephalitis after HCT [116]. It seems to be particularly frequent after cord blood transplant. Cases of encephalitis tend to be accompanied by higher viral loads of HHV-6 in plasma [117], but the role of systematic monitoring of HHV-6 in plasma is unknown at this time, as reactivation seems much more common than disease [118] and attempts to use a preemptive strategy using foscarnet have not been successful [119]. The European Conference on Infections in Leukemia has proposed evidence-based guidelines to address the diagnostic and therapeutic uncertainties related to this infection [120].

6.8.2 Respiratory Viruses

Respiratory viruses, a heterogeneous group of virus that is responsible for most upper acute respiratory infections in normal hosts, result in significant morbidity and mortality after HCT, particularly during the first 3 months following transplant [121]. Even asymptomatic carriage of respiratory viruses at the time of transplant has been reported to result in increased risk of unfavorable outcomes [122]. Besides respiratory syncytial virus (RSV) [123], influenza, parainfluenza virus (PIV) [124], rhinovirus [125], and adenovirus, newly identified viruses including metapneumovirus [126], coronavirus [127], and bocavirus [128] have emerged as significant pathogens. These infections present significant risks both acutely and in the long term. During the acute infection, HCT recipients are at risk of developing viral pneumonia

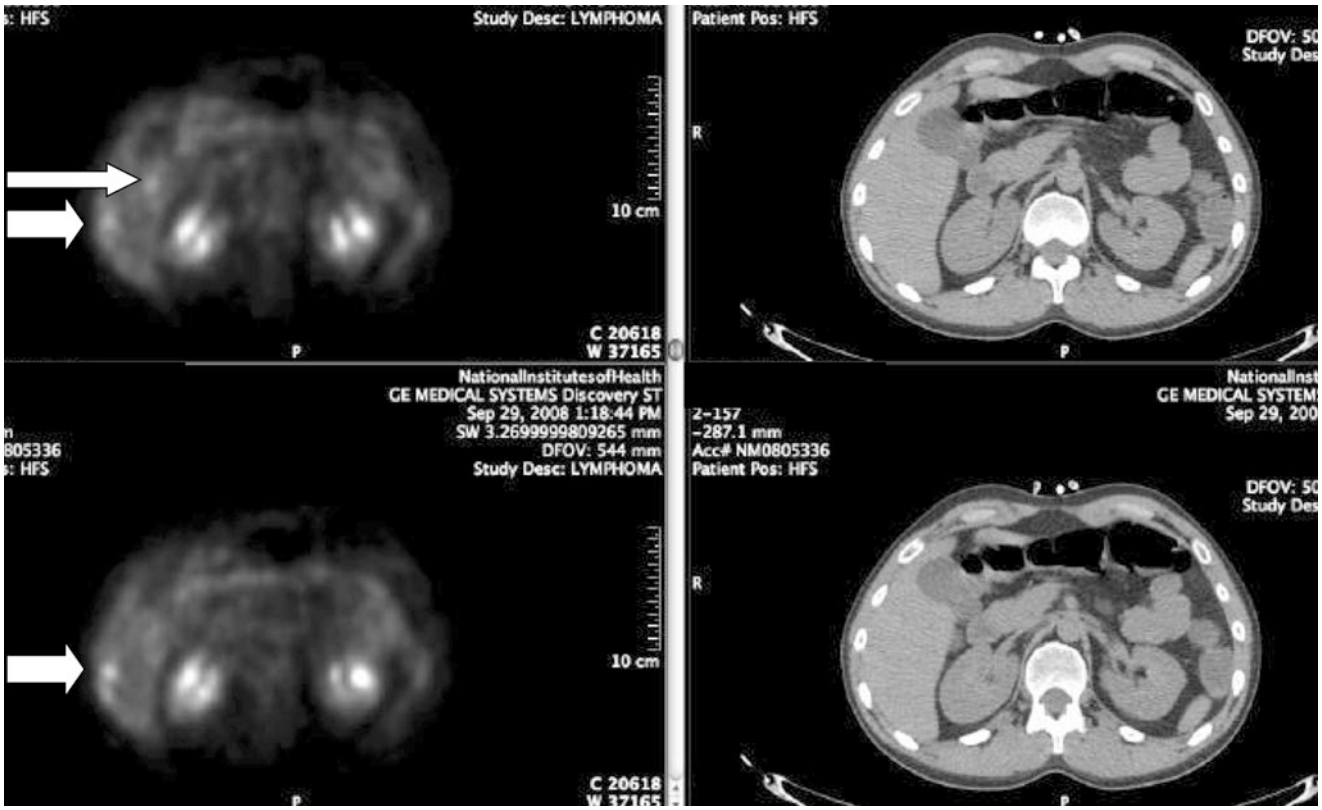

FIGURE 6-2. EBV-related lymphoproliferative disorder after a matched unrelated donor transplant. A 24-year-old man with Hodgkin lymphoma underwent a syngeneic HCT followed by MUD HCT (cyclophosphamide + fludarabine followed by alemtuzumab and cyclosporine). His day-28 CT/PET showed a mixed response: improvement in the intrathoracic lesions and cervical lymph nodes but appearance of new PET+ lesions in the liver, pharynx, and stomach. EBV viral load had been increasing slowly. Biopsies of the PET+ liver and stomach lesions showed a polyclonal EBV+ B cell infiltrate. The disease responded to rituximab and cyclosporine taper.

that sometimes progresses to respiratory insufficiency, mechanical ventilation and death, and also at risk of concomitant or secondary bacterial or fungal infections that are associated with increased mortality [124, 129, 130]. Long-term, there seems to be an association between early infection (pre-day 100) with some of these viruses (most notably PIV and RSV) and later development of chronic airflow obstruction [131]. The most significant risk factor overall for progression of these infections from the upper respiratory tract to the lungs seems to be lymphopenia [132]. Corticosteroid use seems to contribute to progression to pneumonia in RSV and parainfluenza infections but not so in influenza [129, 130] (see Table 6-3).

6.8.3 Adenovirus

Besides its role among the community-acquired respiratory virus, adenovirus may cause disease in transplant recipients following reactivation in the gastrointestinal tract followed by dissemination and end-organ damage [133]. De novo acquisition of adenovirus may also result in disseminated disease. There are more than 60 types of human adenovirus, with different tropisms and possibly varying susceptibilities to antiviral agents. They can cause a variety of diseases, including upper and lower respiratory tract infection, colitis, hemorrhagic cystitis (HC), nephropathy, and CNS disease. Systemic adenovirus disease seems to be more common in children, particularly in recipients of cord blood or T cell-depleted transplants [134–136]. Patients with GVHD on treatment with high-dose corticosteroids are also at risk (Figure 6-3). Some studies have documented that sustained high levels of adenoviremia are associated with disease [137]. It is not known yet whether a preemptive approach with cidofovir can successfully prevent disseminated disease and death [133, 138].

6.8.4 Polyomavirus: BK and JC Virus

6.8.4.1 BK Virus

BK virus infects 90% of humans by age 12. It predictably reactivates in most patients following HCT and causes hemorrhagic cystitis (HC) in a minority of them [139]. Detection of high levels of BK in the peripheral blood seems to correlate with the presence of BK-induced HC [140, 141]. In a

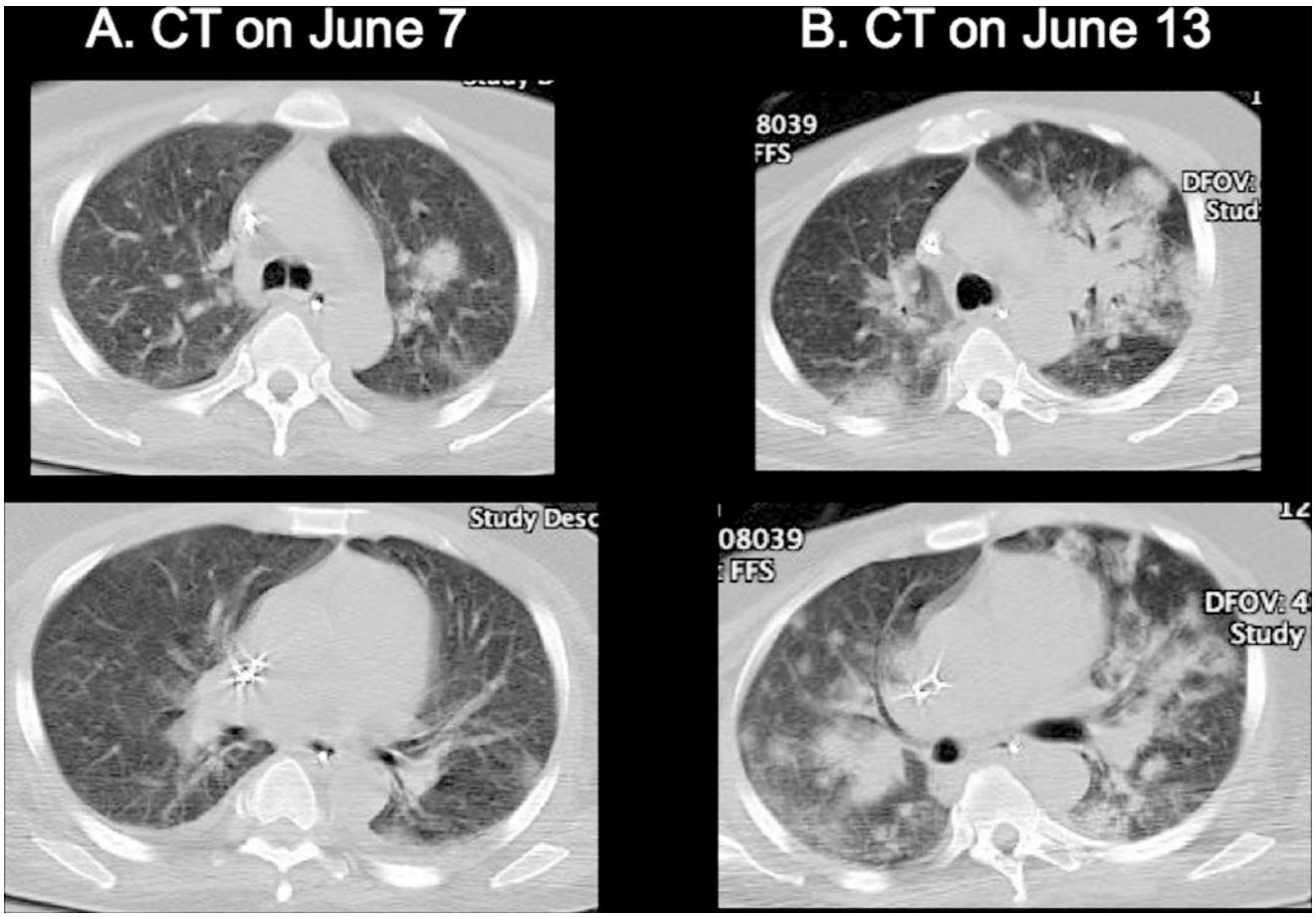

FIGURE 6-3. Adenovirus pneumonia in the setting of disseminated disease. A 48-year-old man received HLA-matched sibling donor nonmyeloablative HCT for myelodysplastic syndrome in transformation. Leukemia recurred immediately after transplant. He received several donor lymphocyte infusions/stem cell boosts and then induction treatment for AML with FLAG (fludarabine + cytarabine + G-CSF) followed by donor stem cells. Graft-versus-host disease involving the skin and gut had been documented being treated with methylprednisolone 1 mg/kg/day. After the patient recovered from neutropenia, he developed spiking fever and progressive shortness of breath. Adenovirus was isolated from tears, respiratory secretions, and urine. PCR in the blood was positive for adenovirus, and the autopsy showed only disseminated adenovirus disease.

large study from the Fred Hutchinson Cancer Research Center (FHCRC), no association was found between BK virus-associated HC and lymphopenia, corticosteroid use, and GVHD—the typical risk factors for viral infections after HCT [140]. In contrast, other smaller studies have found an association with GVHD. The pathogenesis of this disease remains unexplained. BK-induced nephropathy, a common problem after kidney transplant, remains infrequent after HCT and does seem to be related to profound immunosuppression [142]. BK pneumonitis has also been described, but it is distinctly rare [143].

6.8.4.2 JC Virus

JC virus is also acquired by most people during childhood. In immunocompromised hosts, it may cause encephalitis (JC encephalitis, previously called progressive multifocal leukoencephalopathy (PML)) with multiple areas of demyelination without edema detectable by MRI. Some studies have suggested that detectable viral load after HCT may be more common than currently thought [144]. Ascertaining risk factors for this disease is difficult because some transplant recipients may have conditions known to be associated with it and also received medications like MMF, rituximab, or brentuximab, which have been associated with PML even in the absence of allo-HCT.

6.9 Risks and Epidemiology of Pneumocystis After Allogeneic HCT

PCP is an opportunistic infection of patients with profound cellular immunodeficiency, and prophylaxis is recommended after HCT. It is now relatively uncommon: 1.3–2.4% of

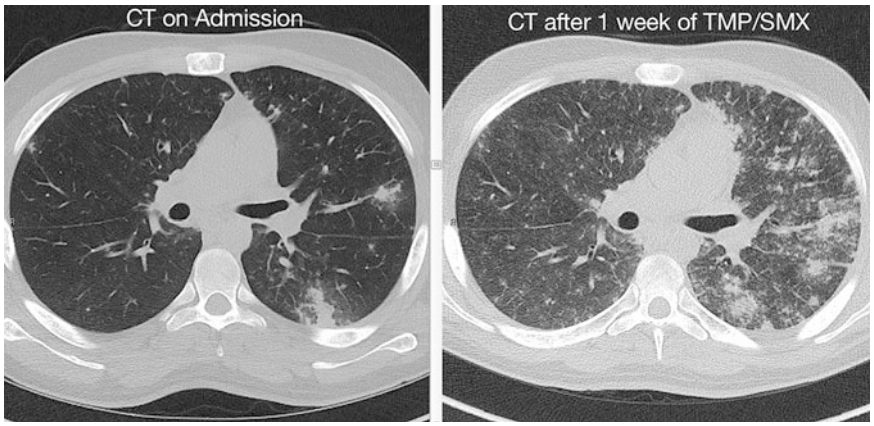

FIGURE 6-4. Pneumocystis pneumonia. A 23-year-old man with Ph+ALL s/p matched sibling allo-HCT presented for his 1-year post-transplant visit complaining of worsening fever and cough over the last 2 weeks, despite oral levofloxacin. He was in complete remission. A month earlier, abnormal liver enzymes had prompted the initiation of sirolimus for suspected chronic GVHD. He was on prophylaxis with acyclovir and atovaquone. The CT showed multifocal infiltrates. The bronchoalveolar lavage showed abundant *Pneumocystis*. After 1 week of treatment with trimethoprim/sulfamethoxazole, the radiographic pattern became characteristic of pneumocystis pneumonia. Atovaquone failures are well documented. The radiographic features of PCP after allogeneic transplant may be atypical.

patients transplanted from several series [145, 146] Most cases seem to occur relatively late, after discontinuing prophylaxis or during periods of intensive immunosuppression for the treatment of GVHD [147]. Hypoxemia is characteristic at presentation. Atypical radiological manifestations, including nodular infiltrates and pleural effusions (in contrast to typical interstitial pneumonitis), are described frequently, as is the presence of co-pathogens [148]. The preferred prophylaxis is trimethoprim/sulfamethoxazole (TMP/SMX), and several dosing regimens are effective (one single-strength tablet daily, one double-strength tablet daily, or one double-strength tablet three times/week) [149]. TMP/SMX may be poorly tolerated because of hematologic toxicity, skin rash and/or gastrointestinal toxicity [150].

It is unclear which is the prophylaxis of choice if TMP/SMX cannot be used. Aerosolized pentamidine is convenient, obviates the problem of compliance, and is less toxic than dapsone and better tolerated than atovaquone. However, it has been reportedly associated with more failures than dapsone [150]. Dapsone seemed to be effective and well tolerated in one study [151] but not in another when it was given only three times per week [152]. Dapsone should not be given to patients with G6PD deficiency. Methemoglobinemia is a well-known complication of dapsone [153] that should be considered in the presence of unexplained shortness of breath. Atovaquone suspension 1500 mg/d may be used, but published experience in HSCT recipients is limited [154, 155]. Atovaquone is expensive and poor tolerance has made compliance for some patients difficult. Absorption is better in the presence of significant amount of fat, and breakthroughs are well documented (Figure 6-4). PCP prophylaxis is recommended at least until all immunosuppression has been stopped but it is unclear how much longer to continue it [156].

6.10 Risks and Epidemiology of Toxoplasmosis After Allogeneic HCT

Most cases of toxoplasmosis after HCT represent reactivation, although rare cases of transmission with bone marrow transplant have been suspected [157]. Recipients should be tested for anti-toxoplasma IgG antibody, and if they are found to be positive, prophylaxis is recommended. Rare cases of toxoplasmosis after HCT have occurred in seronegative recipients [158, 159]. The disease tends to occur within the first 6 months after transplant, but it can happen later in the presence of persistent immunosuppression [160–162]. The risk of toxoplasmosis varies with the type of transplant and the immunosuppression: cord blood and use of ATG were found to be risk factors for disease in a prospective study [162]; most cases in another series occurred in URD or mismatched transplants [107].

TMP/SMX as given for PCP prophylaxis is considered adequate to prevent toxoplasmosis, although there have been cases on HCT recipients who were receiving it [162]. The best alternative for patients who are intolerant to TMP/SMX is unknown. Dapsone and atovaquone showed some efficacy in HIV-infected patients and there is increasing experience after HCT [163], although failures have been reported. Other

regimens include clindamycin with pyrimethamine and leucovorin, pyrimethamine with sulfadiazine, or pyrimethamine and sulfadoxine and leucovorin [107]. If a reliable quantitative PCR assay is available, frequent monitoring and preemptive treatment may be appropriate, since PCR-detected reactivation seems to precede symptoms by 4–16 days [162]. Retrospective data suggest this strategy may result in improved outcome [164].

6.11 Summary

In summary, infections following HCT are frequently related to risk factors caused by the procedure itself. Neutropenia and mucositis predispose to bacterial infections. Prolonged neutropenia increases the likelihood of invasive fungal infection. GVHD and its treatment create the most important easily identifiable risk period for a variety of infectious complications, particularly mold infections. Profound, prolonged T cell immunodeficiency, present after T cell-depleted or cord blood transplants, is the main risk factor for viral problems like disseminated adenovirus disease or EBV-related PTLD.

Besides all these "procedure-related" risk factors, there are individual characteristics that only now are starting to be investigated and understood. Future epidemiological and basic studies will likely result in truly personalized prophylactic regimens that will increase the unquestionable benefits of antimicrobial prophylaxis and reduce the cost, both direct and indirect, associated with this life-saving practice.

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7
Risk and Epidemiology of Infections After Solid Organ Transplantation

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Transplant recipients are uniquely susceptible to infectious diseases due to the nature of their underlying conditions and their immunosuppressed status; consequently infections in solid organ transplantation may be associated with significant morbidity and mortality. These patients are not only vulnerable to a broad range of infectious organisms including those not normally considered to be pathogenic, but also prone to unusual presentations and more severe manifestations of infection [1]. Given the impact of infections on patient morbidity and mortality, it is critical to implement effective strategies for the prevention and early recognition of infectious complications to improve outcomes in this patient population.

This chapter is an introduction to the risks and epidemiology of infections in solid organ transplantation. It provides an overview of pre-transplantation donor and candidate screening, reviews the classic timeline for infection, and discusses methods for disease prevention including immunizations, environmental control, and post-transplantation prophylaxis.

7.1 Pre-transplantation Screening

An essential component of the pre-transplantation evaluation includes screening organ donors and transplant candidates for latent and active infections [2, 3]. This screening process is important for several reasons. Transplant care providers may identify scenarios that warrant exclusion of the organ donor or candidate from transplantation or they may diagnose active infections that require treatment prior to transplantation. The risk of post-transplantation infections that may result from reactivation of latent disease in the setting of increased immunosuppression should also be assessed and strategies individualized to minimize this risk. Lastly, planning preventive measures should be a focus during this period; this includes developing strategies for safe living and immunization, especially since vaccine response after transplantation may be suboptimal.

7.1.1 Screening the Transplant Candidate

All transplant candidates should be screened for latent and active infections using a variety of modalities. It is important to start with a careful history and physical examination. A detailed history including occupational history, places of residence, travel history, pets, and hobbies should be obtained from the transplant candidate. In some cases, this may suggest the presence of active infection and additional evaluation may be indicated to exclude diagnoses that would warrant delaying transplantation (e.g., infections with pathogens for which there are no antimicrobials such as West Nile virus [WNV] or certain respiratory viruses). Alternatively a history of prolonged residence in or birth in a location notable for unique endemic infections may prompt evaluation for subclinical infections that could reactivate after transplantation, including *Strongyloides stercoralis*, *Trypanosoma cruzi*, *Histoplasma capsulatum*, and *Coccidioides immitis* [2, 3]. All transplant candidates should be screened for infectious pathogens that have been more frequently associated with post-transplant complications by using a routine panel of testing, which is then supplemented with additional testing as indicated by history (Table 7-1). This includes serologic testing for cytomegalovirus (CMV), herpes simplex virus (HSV), varicella zoster virus (VZV), Epstein–Barr virus (EBV), human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), syphilis, and *Toxoplasma gondii* [2, 3]. Tuberculin testing is also important due to the increased risk of reactivation of latent infection following transplantation; both intradermal tuberculin purified protein derivative placement and interferon gamma release assays (IGRA) have been used [4, 5].

Pre-transplant screening may reveal active infections that could impact post-transplantation outcomes. Some of these may warrant excluding the transplant candidate from solid organ transplantation either temporarily or definitively. In some cases, such as WNV infection, transplantation may occur following resolution of the infection. In other cases, patients may not be eligible either due to the severe or incurable nature of the infection. Notably these criteria have

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Table 7-1. Infectious diseases screening for solid organ screening for solid organ transplant candidates

Routinely administered tests	Other potential tests based on history; or clinical, laboratory, or radiologic findings
CMV antibody	Blood cultures[a]
EBV antibody (EBV VCA IgG and/or EBNA[b])	Chest imaging
HBV (hepatitis B surface antigen and antibody, and core antibody)	*Coccidioides* antibody[c]
HCV antibody and HCV RNA[d]	Cryptococcal antigen[a]
HIV 1/2 antibody	*Histoplasma* antibody[c]
HSV antibody	Respiratory virus PCR panel (including influenza A and B, parainfluenza, RSV, metapneumovirus, adenovirus)[a]
PPD or IGRA (interferon gamma release antibody)	*Schistosoma* antibody[c]
RPR (rapid plasma reagin)	*Strongyloides* antibody[c]
Toxoplasma antibody	*Trypanosoma cruzi* antibody[c]
VZV antibody	Urinalysis and urine culture
	WNV (West Nile Virus) antibody or nucleic acid testing[a,c]
	HTLV I/II antibody

[a]Recommended for individuals with symptoms consistent with these infections.
[b]*VCA* viral capsid antibody, *EBNA* Epstein–Barr nuclear antibody.
[c]Recommended for individuals from geographic regions where these infections are endemic.
[d]Recommended for candidates who are immunosuppressed prior to transplant, including those with end-stage renal disease.

evolved over time and infections that were once considered exclusions to transplantation may now be acceptable. One example of this is HIV infection, previously considered a contraindication due to concerns that immunosuppressive medications would increase the risk of opportunistic infection, HIV disease progression, and mortality [6]. The advent of highly active antiretroviral therapy (HAART) has allowed for outstanding long-term HIV control in adherent patients, and the excellent outcomes in HIV-infected individuals undergoing liver and especially kidney transplantation have encouraged increasing numbers of centers to perform renal and hepatic transplants in these patients [7–12]. Centers are also beginning to consider thoracic and pancreatic organ transplantation in HIV-positive individuals given acceptable outcomes in the initial patients undergoing these procedures [13–16]. Another exception may be infection of ventricular assist devices, as eradication of these infections typically requires device explantation and outcomes have been acceptable with transplantation [17, 18]. Many transplant candidates are hospitalized with high acuities of illness, requiring invasive procedures (e.g., central line placement, intubation, or ventricular assist device insertion) that place them at increased risk of infection. These patients require close monitoring in order to promptly identify active infections that occur while awaiting transplant; ideally these should be adequately treated prior to transplantation to minimize post-transplant complications.

Pre-transplantation screening can also identify latent infections that may reactivate after transplantation. Possible pathogens include bacteria (e.g., *Mycobacterium tuberculosis*), viruses (e.g., CMV, HBV, HCV, BK virus, EBV, HSV, and VZV), and fungal pathogens (e.g., *Histoplasma capsulatum and Coccidioides immitis*). The risk for TB is significantly increased in the transplant population with most cases representing reactivation of latent infection and screening should be performed in all transplant candidates. Options include intradermal skin testing utilizing PPD or IGRA with the latter preferred in individuals who have received BCG immunization to reduce the rates of false-positive tests [5]. When PPD testing is performed, areas of induration ≥5 mm are considered positive and patients with negative PPD results on initial testing may be considered for repeat testing after 2 weeks [5]. Both PPD and IGRA have reduced sensitivity due to common acquired immune deficiencies in candidates for transplantation; consequently it is important to take a careful history and closely review chest radiography in high-risk individuals who have negative testing. It is also important to scrutinize individuals with discordant test results. Treatment is recommended for all transplant candidates with a positive tuberculin test after active disease has been excluded. Since many transplant candidates are anergic, high-risk patients with a negative PPD or an indeterminate IGRA should also be considered for empiric treatment. These include transplant recipients with radiographic evidence of prior disease, a history of inadequately treated TB, close contact with someone who has active TB, or receipt of an organ from an inadequately treated organ donor [5, 19]. First-line treatment involves a 9-month course of isoniazid. Those who are isoniazid intolerant may benefit from a 4-month course of rifampin. However, rifampin has multiple drug interactions, including increasing the metabolism of calcineurin inhibitors, which may significantly complicate its administration post-transplantation. Consequently its use should be carefully considered in patients who may be transplanted during the course of therapy. In some cases, fluoroquinolones may be considered as alternate therapy for latent tuberculosis [20].

The optimal timing of latent TB treatment has not been defined and varies depending on the patient's risk for treatment-related hepatotoxicity. Patients without liver disease often undergo treatment prior to transplantation [5]. Because the risk of hepatotoxicity is increased in liver transplant candidates, transplant centers may choose to initiate latent TB treatment after transplantation once the patient is stable and the liver function tests have normalized [21]. Of note, treatment can be ongoing at the time of transplantation. Given that the highest risk of reactivation is within the first year following transplantation, if patients cannot be treated prior to transplantation, it is preferable to treat them as soon

as possible following transplantation [4]. Recent converters should be treated prior to transplantation if at all possible to decrease the risk of active infection either prior to or at the time of transplant.

Transplant candidate screening is an important opportunity for reevaluation of standard preventive measures. This includes an assessment of their home and work environments, pets, and hobbies in order to implement sufficient preventive measures prior to transplantation. The pre-transplant evaluation period is also an important opportunity for updating routine immunizations for vaccine-preventable illnesses, especially influenza and pneumococcal infection [22]. Patients should also undergo assessment for immunity to vaccine-preventable illnesses (e.g., varicella and hepatitis A and B) and be immunized accordingly. Further recommendations regarding immunizations will be included later in this chapter.

7.1.2 Screening the Organ Donor

The ability to fully screen organ donors will vary depending on whether the donor is living or deceased. Living donor screening has certain advantages. Notably the donor provides his or her own medical history and testing may be tailored to risks identified during the initial donor assessment. In contrast, deceased donor screening is limited to several hours where the medical history is obtained from a proxy. Family members may provide incomplete or inaccurate medical history and may be unaware of patient behaviors that place the patient at higher risk of infection. Despite these differences, the infectious pathogens tested during donor screening are similar for living and deceased donors and mirror the testing done in transplant candidates, although some testing may be performed in live donors that is currently not available or considered to be reliable in deceased donors (e.g., testing for tuberculosis). Potential donors are tested for a panel of routine serologies with additional screening guided by pertinent patient history. Further detail regarding screening the organ donor is provided in Chap. 8.

7.2 The Risk of Infection Posttransplantation

The risk of infection post-transplantation is determined by the balance between the patient's net state of immunosuppression and his or her epidemiologic exposures [1]. A patient who is more severely immunosuppressed may be susceptible to a broader range of infections, including those caused by opportunistic pathogens. Although opportunistic pathogens are more likely to cause infection in the first 6 months after transplantation, when patients are expected to be more immunosuppressed, patients in the late post-transplantation period (>6 months) on minimal immunosuppression without a significant history of rejection may be at risk of opportunistic infection, especially following a significant epidemiologic exposure.

Multiple factors contribute to a transplant recipient's net state of immunosuppression. The type, dosage, and timing of administered immunosuppressive medications remain the primary factor. Different immunosuppressive medications exert unique effects on the host immune system: (1) corticosteroids inhibit inflammatory responses and affect T-cell activation, (2) cytotoxic agents (i.e., azathioprine and mycophenolate acid) impair T-cell and B-cell proliferation and function, (3) calcineurin inhibitors (i.e., tacrolimus and cyclosporine) inhibit cytokine production, primarily interleukin-2 (IL-2), by CD4-positive T-cells, (4) target of rapamycin (mTOR) inhibitors (i.e., sirolimus) inhibit cell cycle proliferation and are associated with delayed wound healing and oral ulcers, (5) monoclonal antibodies (i.e., basiliximab and daclizumab) target the IL-2 receptor, (6) recombinant monoclonal antibodies (i.e., alemtuzumab) bind to CD52 on B and T lymphocytes, a majority of monocytes, macrophages, and NK cells, and a subpopulation of granulocytes, disrupting these cellular functions for prolonged periods, (7) polyclonal antibodies (i.e., antithymocyte globulin) induce lysis of lymphocytes with prolonged lymphocyte depletion increasing the risk of infection 3 months or longer after administration, and (8) costimulatory blockade agents (i.e., belatacept) disrupt T-cell costimulation and consequently activation. Immunomodulatory viruses (i.e., CMV, EBV, HBV, HCV, and HIV) and existing comorbidities (e.g., diabetes, renal insufficiency, and malnutrition) also contribute to the net state of immunosuppression. Transplant recipients often have more than one factor present, resulting in defects in multiple arms of the immune system. Although tests are available to assess certain immunologic defects (e.g., quantitative immunoglobulins, lymphocyte subset measurements, and gamma interferon release assays targeted against specific pathogens), there is no single test currently available that accurately assesses this net state of immunosuppression.

Epidemiologic exposure is the other main determinant of a transplant recipient's risk of infection. This exposure can be nosocomial or community acquired and may have occurred prior to transplantation. Latent infections can reactivate after transplantation in the setting of enhanced immunosuppression. Different organs also have unique epidemiologic risks due to organ-specific surgical procedures and environmental factors. For example, transplanted lungs may be at increased risk for colonization and/or infection with inhaled pathogens due to decreased mucociliary function, ischemia at the anastomosis site, and the direct exposure of the transplanted organ to the external environment. Liver and pancreas transplant recipients may be at risk of infection related to a particular surgical method (e.g., bowel anastomosis), while heart transplant candidates with pre-transplant ventricular assist devices may be at risk for device-related infections.

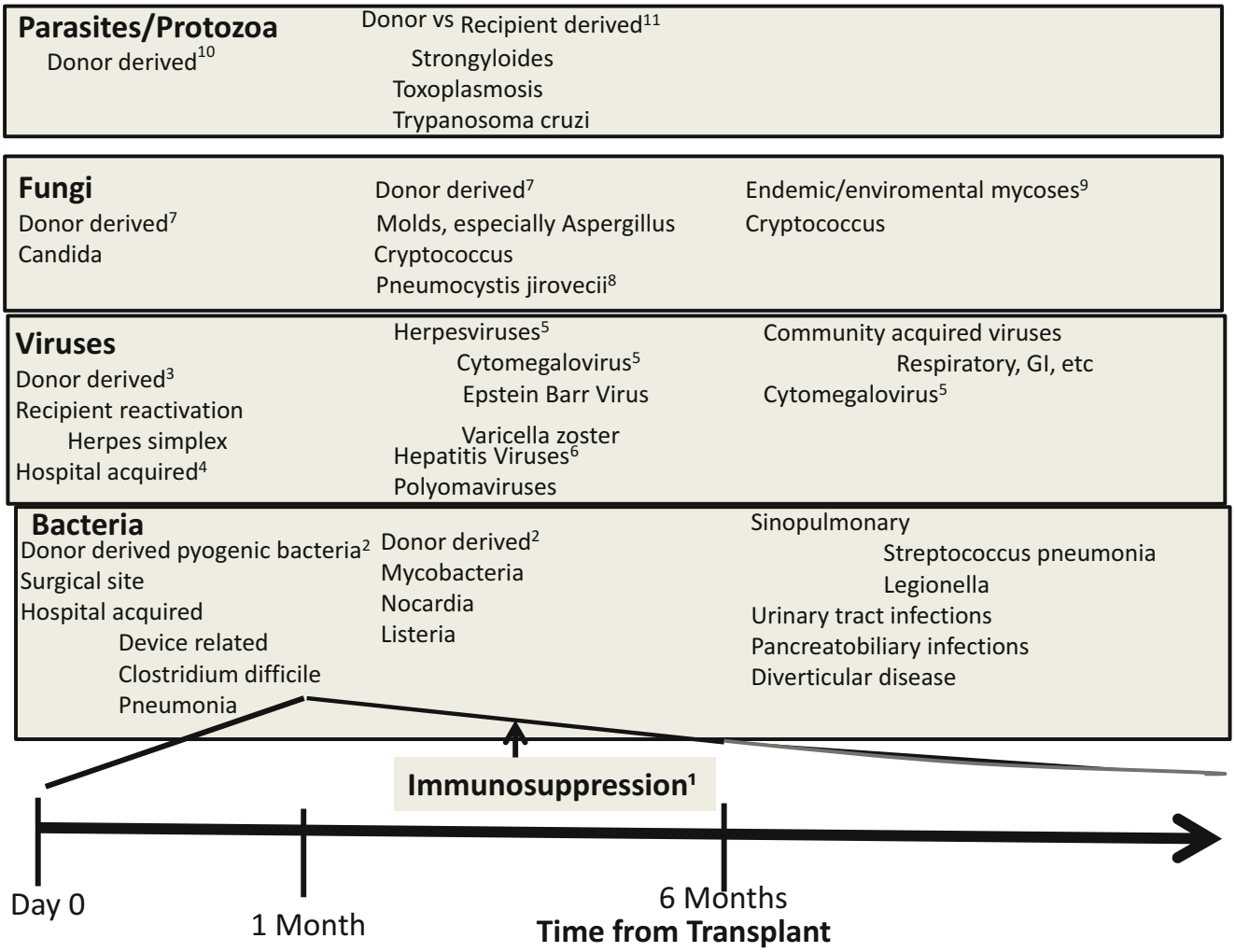

FIGURE 7-1. Epidemiology of post-transplant infection considering time from transplant. Adapted from Fishman JA, Rubin RH, Infection in organ transplant recipients, New England Journal of Medicine, 1998;338(24):1741–51, and Fishman JA, Infection in solid organ transplant recipients, New England Journal of Medicine, 2007;357(25):2601–14.

Standardized immunosuppressive therapy in solid organ transplantation has enabled the development of a useful predictive timeline for infection [1]. It includes three distinct time periods: the early post-transplantation period (0–1 month), the intermediate post-transplantation period (1–6 months), and the late post-transplantation period (>6 months) (Figure 7-1). Although changes in immunosuppressive medication regimens combined with the use of antimicrobial prophylaxis have modified this timeline, the general framework is still applicable. Transplant care providers can use this reference to develop an initial differential diagnoses for transplant recipients who present with signs of infection as well as to devise prophylactic strategies [1, 23]. However it is important to recognize that exceptions are possible; patients receiving certain more long-lasting immunosuppressive agents and those with chronic rejection or infection with immunomodulatory viruses may remain severely immunosuppressed and therefore be more vulnerable to opportunistic pathogens beyond 6 months after transplantation. Additionally it is possible that patients (especially pediatric recipients) may acquire primary infection with common opportunists (e.g., CMV and EBV) in the later post-transplant period, thus delaying the presentation of these infections.

7.2.1 Risk of Infection in the Early Posttransplantation Period (0–1 Month)

Within the first month, transplant recipients are most susceptible to nosocomial infections similar to those seen in non-immunosuppressed surgical patients, including pneumonia, urinary tract infections, catheter-related blood stream infections, and surgical site infections [3, 8, 10, 24]. Some of these may be directly related to complications of the surgical

procedure or a consequence of postoperative care; bacteria and *Candida* species are most commonly implicated [23]. Liver transplant recipients, for example, may be at increased risk of wound infections, or infections such as peritonitis or abdominal abscesses due to leaks at the biliary anastomotic site. The postoperative care may involve prolonged periods of intubation and the insertion of central venous lines or indwelling urinary catheters. These breaks in the mucocutaneous barriers place patients at increased risk for nosocomial infections including ventilator-associated pneumonias, surgical site infections, bloodstream infections, or urinary tract infections. Perioperatively, patients often receive broad spectrum antibiotics that may contribute to the emergence of antimicrobial-resistant pathogens, nosocomial fungal infections, and infection with *Clostridium difficile* colitis. Nosocomial transmission of viruses, especially respiratory viruses, may also occur [25]. Immunocompromised patients may have longer periods of viral shedding, and therefore they have opportunities to infect other organ transplant recipients by being placed in adjacent rooms on dedicated wards. Additionally, infection with *Legionella* species can occur anytime including the early post-transplantation period [26, 27]. Pneumonias are among the most commonly seen early infections; however not all pulmonary infiltrates are due to infection. The differential diagnosis is broad, and may include edema, atelectasis, rejection (in lung transplantation), and medications in addition to infection [28]; it is important to consider these diverse possibilities when evaluating patients. Transplant recipients are at increased risk for more severe pneumonias, including cavitary pneumonias; consequently early recognition of infection is critical [28–30].

Opportunistic infections are usually absent during the first month after transplantation, although there have been rare reports of infection with these pathogens. Among the most commonly reported is Aspergillus pneumonia (especially in lung recipients) [24]. It is probable that this low incidence can be attributed to the delayed impact of immunosuppressive medications introduced during this period.

Although the vast majority of early infections are nosocomial, a low incidence of donor and recipient-derived infections may also occur during this period. A Spanish study evaluating nonviral donor-derived infections reported disease transmission in 5/292 (1.71 %) of transplant recipients who received organs from infected donors [31]. All five donor-derived infections were due to bacterial pathogens. In the United States, there is mandated reporting of suspected donor-derived infections to the United Network for Organ Sharing (UNOS). Reviews of these reports by the Disease Transmission Advisory Committee of UNOS have noted that diverse pathogens have been transmitted including pyogenic bacteria, *T. cruzi*, HCV, HIV, WNV, lymphocytic choriomeningitis virus (LCMV), *Legionella* species, *H. capsulatum*, *Candida* species, *S. stercoralis*, *C. neoformans*, *Schistosoma* species, *T. gondii*, and *M. tuberculosis* [32, 33]. The timing of these infections varies; typically bacterial infections and fungal infections are most likely to present in the first month [34]. Although uncommon, these unanticipated donor-derived infections have been associated with increased morbidity and mortality, with complications including surgical site infections and mycotic aneurysms [32, 33, 35–37]. The transmission of more unusual infections in solid organ transplantation, including WNV, LCMV, or rabies, has also been documented [38–40]. Diagnosis of these infections may be confounded by the absence of recognized donor infection and the presentation of nonspecific symptoms such as fever, altered mental status, or liver enzyme elevations, followed by a rapid clinical decline oftentimes resulting in death. Recent reports have cautioned against the use of donors with neurologic processes of unclear etiology to prevent transmission of some of these infections [41].

Early infections may also occur as a continuation of an active infection in the recipient that precedes transplantation. Not all of these may be recognized prior to transplantation. Pathogens related to early recipient-derived infections may be diverse; occasionally opportunistic pathogens may occur, especially in liver transplant patients or those treated with immunosuppressive agents prior to transplantation.

7.2.2 Risk of Infection in the Intermediate Posttransplantation Period (1–6 Months)

The intermediate post-transplantation period generally refers to the time occurring 1–6 months following transplantation. During this period, most solid organ transplant recipients are at their highest net state of immunosuppression due to immunosuppressive medications exerting their full effect. Patients may also develop infections with immunomodulating viruses such as CMV, EBV, HBV, or HCV as well as experience metabolic complications including diabetes and renal insufficiency that can alter the immune system.

During this period, in the absence of prophylaxis, infections due to diverse opportunistic pathogens including bacteria (e.g., *Nocardia species*, *Listeria monocytogenes*, and Mycobacteria), fungi (e.g., *Pneumocystis jiroveci*, *Aspergillus* species, *C. neoformans*), viruses (e.g., CMV, EBV), and parasites/protozoa (e.g., *Toxoplasma gondii*) can occur. Geography can place individuals at risk for endemic mycoses including *Histoplasma capsulatum*, *Coccidioides immitis*, or *Blastomyces dermatitidis*. Lastly, donor-derived infections may present during this intermediate post-transplantation period; this is especially true of parasites (including *Strongyloides stercoralis*), protozoa, and viral infections [34]. Environmental exposures may also play a role during this period and place patients at higher risk of opportunistic infection. This includes exposure to a specific environment as well as to individuals with potentially communicable diseases. The changing nature of immunosuppressive regimens and

prophylactic strategies continues to have an impact on the infections seen during this period; both need to be considered when patients are evaluated. Specific infections seen during this high-risk period are detailed in later chapters.

7.2.3 Risk of Infection in the Late Posttransplantation Period (>6 Months)

The nature of infections in the late post-transplantation period may be a window into the transplant recipient's net state of immunosuppression. This net state of immunosuppression can be underestimated by simply reviewing the medication doses and levels as patient responses to specific regimens may vary substantially. By 6 months after transplantation, the vast majority of transplant recipients are doing well with good allograft function maintained with minimal immunosuppression. These patients typically reside in their home environments where they are most susceptible to community-acquired infections.

Transplant recipients can be at increased risk for more severe respiratory infections with community-acquired pathogens (e.g., influenza, parainfluenza, respiratory syncytial virus, adenovirus, human metapneumovirus, *Legionella* species, and pneumococcus). These patients may have longer durations of both infection and shedding, increased progression to lower respiratory tract infections, higher mortality rates, and increased risk for rejection [27, 42–47]. Transplant recipients are also at a 12.8-fold greater risk for invasive pneumococcal disease compared to the general population [46].

Other viral infections can also be seen during this period, including infections that may have been previously suppressed by prophylactic strategies. CMV infection is the most common opportunistic infection during this period. Often donor derived, CMV that may present variably as prophylaxis is discontinued [3, 48]. Transplant recipients with CMV syndrome have nonspecific symptoms including fever and fatigue that may be accompanied by laboratory abnormalities, most notably leukopenia. CMV may also present with end-organ involvement such as colitis, pneumonitis, or infected allograft. In addition to its direct effect, CMV has indirect effects on the immune system that increase the risk for allograft rejection and loss, post-transplantation lymphoproliferative disorder (PTLD), other infections, and diabetes mellitus [49–55]. Chronic viral infections, such as HBV or HCV, may also reemerge during this time. Outcomes in HBV-infected recipients have improved with the use of HBV immunoglobulin and antiviral agents [56]. Currently HCV-positive recipients fare worse, developing repeated episodes of chronic rejection, post-transplant diabetes mellitus, or chronic infection and end-stage liver disease, although the advent of more effective and better-tolerated antiviral treatment options will likely improve outcomes [56–59]. EBV may increase the risk of infections and malignancy, particularly PTLD. PTLD risk is greatest in EBV-seronegative recipients who receive organs from seropositive donors, a scenario which most often occurs in children [60]. Other risk factors have included the organ transplanted and the choice of immunosuppression agent (especially belatacept) [60–62]. Additionally, EBV viremia may provide insight into a patient's net state of immunosuppression; consequently when routine monitoring detects viremia, immunosuppressive therapy is typically reduced [60, 63].

There are documented cases of reactivation of latent infections during this period. For example, *H. capsulatum* can establish latency after primary infection and reactivate months after transplantation [64]. Posttransplantation TB rates range from 0.35 to 15 % worldwide, depending on disease prevalence with a median time to onset of 6–11 months [5, 65, 66]. The frequency of active TB varies based on the organ transplanted but is substantially higher than that of the general population (20–74 % higher), with most cases resulting from reactivation of latent recipient infection [5, 65, 66]. Donor-derived TB is estimated to account for <5 % cases [5, 67].

Lastly, transplant patients may be at risk of other infections not necessarily associated with immunosuppression. This could include health care-associated infections during periods of hospitalization. Studies suggest that transplant recipients, particularly heart, lung, and heart–lung recipients, may also be at an increased risk of pancreaticobiliary disease and diverticulitis [68, 69].

Although most recipients have stable allograft status and are infection free by 6 months after transplantation, a minority (approximately 20 %) may be chronically infected with immunomodulatory viruses or have recurrent episodes or chronic rejection requiring high-dose immunosuppressive therapy. The classic timeline for infections is not applicable to this subpopulation. These patients are more severely immunosuppressed and continue to be at risk for opportunistic infections well past 6 months post-transplantation, thus potentially making them candidates for prolonged prophylactic strategies.

7.3 Prevention of Infection

Prevention of infection is vital for improving outcomes in the solid organ transplant population. Transplant recipients need to be updated on their immunizations, preferably prior to transplantation, and they should be educated regarding behaviors that can minimize their day-to-day risk. Transplant care providers should also evaluate and identify patients who would benefit from antimicrobial prophylaxis or preemptive therapy when appropriate.

7.3.1 Immunizations

Screening of candidates prior to transplantation is an ideal opportunity to ensure that transplant recipients are updated on the following immunizations, according to age- and

condition-related guidelines: *Streptococcus pneumoniae*, *Haemophilus influenza*, influenza, diphtheria, pertussis, tetanus, hepatitis A virus, HBV, measles, mumps, rubella, poliomyelitis, and VZV [47, 48]. Although highly recommended, there is suboptimal utilization of immunizations in this population. One study reported that only 62.4 % (95 % CI 54.8–70.1 %) of lung transplant candidates received *S. pneumoniae* vaccination [70].

There are several general guidelines regarding the administration of immunizations in this population. First, it is preferable to update immunizations prior to transplantation rather than in the post-transplant period [3, 22]. Studies suggest that transplant recipients have reduced responses to diverse vaccines compared to immunocompetent individuals; this makes pre-transplantation immunization especially important [22, 71, 72]. Second, not only should immunizations be administered pre-transplantation, but they are most effective when administered earlier in the course of disease [73]. Given the suboptimal response to immunizations in certain patients with end-stage organ disease, physicians may consider confirming vaccine efficacy in patients who report prior immunization. Serologic testing for hepatitis A virus, HBV, VZV, measles, mumps, and rubella can be performed to ensure that patients maintain adequate levels of protection [74].

Even after transplantation, patients should ensure that they are current on their immunizations. Typically, immunizations are not given until at least 3–6 months post-transplantation when immunosuppression is reduced sufficiently to allow for improved immune response, although there may be exceptions to this, especially during epidemics (e.g., influenza) [22]. Transplant recipients as well as their family members and close contacts should receive yearly influenza vaccination. Strategies for pneumococcal vaccination include using conjugate pneumococcal vaccine followed by polysaccharide capsule vaccine [75]. Live attenuated vaccinations, including intranasal influenza, VZV, and MMR, are generally avoided in transplant recipients due to an increased risk for possible dissemination; however recent reports suggest that Varicella zoster vaccine may be safe in at least some pediatric recipients [22, 76].

7.3.2 Avoidance of Infectious Exposures

There are certain measures that can minimize the risk of infection among transplant recipients. In the hospital setting, patients should adhere to basic infection control measures. This includes washing hands frequently and limiting exposure to sick visitors and staff. When transplant recipients return home, these basic infection control measures need to be augmented with avoidance of potential environmental hazards. Transplant recipients should watch their diet, avoiding untreated water, undercooked meats, unwashed produce, and unpasteurized dairy products, soft cheeses, and juices. Other preventive measures include circumventing areas undergoing active construction, refraining from changing litter boxes, engaging in safe sexual practices including using latex condoms in non-monogamous sexual contacts or during periods of increased immunosuppression, and limiting hobbies such as gardening that may put them at risk for novel infectious pathogens [77, 78].

7.3.3 Prophylaxis

Prophylactic strategies following transplantation have also included the administration of anti-infective agents for more common or potentially serious pathogens during high-risk periods (Table 7-2). Prior to the initiation of prophylaxis, approximately 10–12 % patients developed PCP infection 2–6 months post-transplantation [1]. Most centers, therefore, provide PCP prophylaxis to all their transplant recipients for at least the first 6–12 months, using trimethoprim-sulfamethoxazole (TMP-SMZ) as first-line therapy. TMP-SMZ not only provides excellent protection against PCP but, when given daily, may provide protection against urinary tract infections in renal transplant recipients, *T. gondii*, *Nocardia* species, and *L. monocytogenes* [78–80]. TMP-SMZ is inexpensive and usually well tolerated.

For patients who are unable to tolerate TMP-SMZ, second-line agents include dapsone, atovaquone, inhaled pentamidine, and a combination of clindamycin and pyrimethamine [81]. These alternatives, however, may not be as effective against PCP and do not provide equivalent protection against additional pathogens like TMP-SMZ [3]. If second-line therapy with dapsone is needed, it is recommended that glucose-6-phosphate dehydrogenase (G6PD) levels should be checked prior to administration since hemolytic anemia and methemoglobinemia may occur at higher rates in transplant recipients compared to HIV patients. However, some studies suggest that these complications may occur even in the setting of normal G6PD levels [82, 83]. The duration of prophylaxis varies. Renal, heart, and liver transplant recipients on routine immunosuppression typically discontinue PCP prophylaxis at 6 months to 1-year post-transplantation, while lung transplant recipients and small bowel recipients, who are at higher risk, typically remain on lifelong prophylaxis [81]. Physicians should also consider reinitiating prophylaxis during periods of increased immunosuppression (i.e., episodes of rejection) or prolonging prophylaxis for patients with chronic rejection.

One of the most important infections in solid organ transplantation is caused by CMV. The risk for infection is predicted by donor and recipient serostatus and varies depending on the organ transplanted and the choice of immunosuppression [48]. The highest risk occurs when a seronegative recipient receives an organ from a seropositive donor (D+ R−). This not only places the transplant recipients at 60–75 % risk of primary CMV infection but also at increased

TABLE 7-2. Prophylaxis frequently administered after solid organ transplantation

Indication		Medication and dose[a]	Duration
CMV[b]		Valganciclovir 900 orally mg once daily	3–12 months[b]
		Ganciclovir 5 mg/kg intravenous administration daily	
		Valacyclovir 2 g orally 4 times daily	
Fungal[c]		Nystatin 5 mL swish and swallow four times a day	At least 1 week–3 months (all)
		Fluconazole 400 mg orally daily	Variable
		Voriconazole 400 mg orally twice daily × 2 doses, then 200 mg twice daily	
		Amphotericin 10 mg/mL inhaled daily (lung recipients)	
Pneumocystis jirovecii	Preferred	TMP/SMZ SS orally daily or DS orally three times a week	6–12 months for non-lung transplant recipients (longer if chronic rejection issues)
		TMP/SMZ SS orally daily	Lung transplant recipients remain on lifelong prophylaxis
	Sulfa allergic	Dapsone 100 mg orally daily Atovaquone	6–12 months for non-lung transplant recipients (longer if chronic rejection issues)
		1500 mg orally daily Aerosolized pentamidine	Lung transplant recipients remain on lifelong prophylaxis
		300 mg inhaled monthly	
Tuberculin test positive without active infection[d]		Isoniazid (INH) 300 mg orally daily and pyridoxine 50 mg orally daily[c]	9 months

[a]Assumes normal renal function.
[b]CMV prophylaxis varies based on the organ transplanted and donor and recipient CMV status.
[c]Guidelines for antifungal prophylaxis vary with organ, the presence or absence of specific risk factors, and the choice of immunosuppression. See Chaps. 39 and 41 for more details.
[d]Timing of treatment of latent infection with respect to transplant may vary depending on the type of organ transplant and whether the patient is a recent converter. An alternative to INH is 4 months of rifampin if given prior to transplantation. Fluoroquinolone-based regimens may be considered for patients with hepatotoxicity from INH or rifampin.

risk of infection with ganciclovir-resistant CMV and possibly recurrent CMV [84, 85]. The risk of infection is significantly lower when the recipient is CMV seropositive. In addition, CMV risk varies based on the organ transplanted. Lung, small intestine, and pancreas transplant recipients are at the highest risk of CMV infection when compared with kidney transplant recipients who are at the lowest risk [48].

Two different strategies, universal prophylaxis versus preemptive therapy, are typically used for CMV prevention [68, 86]. In universal prophylaxis, an antiviral agent is administered to all transplant recipients to prevent the development of CMV infection. In contrast, preemptive therapy involves close surveillance of CMV viral shedding (typically in the blood) in transplant recipients with therapy initiated when positive levels are detected. CMV DNA testing has supplanted antigen testing at most transplant centers. Meta-analyses suggest that compared to preemptive therapy, universal prophylaxis is associated with decreased rates of allograft rejection, opportunistic infections, and mortality [87, 88]. Randomized controlled trials in kidney transplant recipients have reported lower rates of graft loss [89] and lower rates of acute rejection among patients in the universal prophylaxis arm [90] but an increase in late CMV infection [91], higher medication costs, and increased medication-related toxicity [86].

Antiviral agents used for CMV prophylaxis include ganciclovir, valacyclovir, and valganciclovir. Valganciclovir, a prodrug of ganciclovir, administered once daily has become increasingly popular for prophylaxis in transplant recipients. The use of valganciclovir, however, may be less effective in liver transplant recipients, where there may be a higher incidence of tissue-invasive disease [92]. CMV hyperimmunoglobulin is used infrequently for CMV prophylaxis. One meta-analysis reported no differences between CMV disease, infection, or all-cause mortality in patients who received prophylaxis with an antiviral alone or in combination with CMV immunoglobulin [93]. Another meta-analysis reported that while the rates of CMV infection and rejection did not differ between groups, those that received CMV immunoglobulin had lower rates of CMV disease, overall mortality, and CMV-related mortality [94]. Duration of prophylaxis varies but is given for at least 90 days post-

transplantation, with longer durations for CMV-seronegative recipients of seropositive donor organs and for lung recipients [86]. Transplant care providers should consider reinitiating prophylaxis during episodes of rejection necessitating enhanced immunosuppressant therapy, particularly with antilymphocyte antibodies [48].

Fungal infections are associated with significant complications in the solid organ transplant population. Overall, the most common cause of invasive fungal infections is *Candida* species, followed by *Aspergillus* species [95]. Data from the Transplant Associated Infection Surveillance Network (TRANSNET) reported that the most common *Candida* species was *Candida albicans*, followed by *Candida glabrata*, which has a higher rate of fluconazole resistance [95]. Liver transplant recipients are particularly prone to invasive candidiasis, especially if they have two or more of the following classic risk factors: prolonged operation time, high transfusion requirements (>40 units of blood products), Roux-en-Y biliary anastomosis, renal insufficiency (preoperative serum creatinine > 2 mg/dL), re-transplantation or reoperation, and colonization with *Candida* species [96]. Potential risk factors that include further validation include antibiotic prophylaxis with a fluoroquinolone for spontaneous bacterial peritonitis and patients with iron overload [97]. Among all transplant recipients, lung transplant recipients appear to be at highest risk for invasive fungal infections with organisms other than with *Candida* species [95]. This population is at increased risk for infection with *Aspergillus* species, particularly at the site of anastomosis. Although voriconazole or aerosolized amphotericin B is often used, there are no large-scale, multicenter, randomized studies to direct guidelines regarding the role of antifungal prophylaxis in this population. Nevertheless many centers favor the use of antifungal prophylaxis especially for patients with risk factors including airway ischemia, *Aspergillus* colonization, CMV infection, and augmented immunosuppression [98].

Despite conclusive data to support the use of prophylactic antifungal agents, most transplant centers choose to provide antifungal prophylaxis to certain transplant recipients who are at the highest risk of invasive fungal infections (e.g., liver transplant recipients with the aforementioned risk factors and lung transplant recipients).

7.4 Summary

Infections are serious complications of solid organ transplantation that are largely determined by two factors: the transplant recipient's net state of immunosuppression and the epidemiologic exposures (including those in the pre-, peri-, and post-transplant settings). Diagnosis and management of infections in this population may be challenging. The recipient's immunosuppressed state not only makes him or her susceptible to a broad range of infectious pathogens but may also alter the presentation and affect treatment and outcomes. Given the significant morbidity and mortality associated with infections, preventive measures as well as early diagnosis and treatment are vital in improving outcomes in this patient population.

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8
Donor-Derived Infections: Incidence, Prevention, and Management

Nicole Theodoropoulos and Michael G. Ison

8.1 Introduction

Solid organ transplantation is an ever evolving field, with significant advances in the management of recipients of solid organ transplantation, including enhanced immune suppression and antimicrobial prophylaxis for at-risk patients [1, 2]. In order to increase the number of available organs, and, in turn, save more lives of those on the transplant wait list, the donor pool must continue to expand [3]. Donors reflect the diverse US population; there are an increasing number of donors born in, who have resided in, or who have traveled to underdeveloped areas of the world or areas with geographically restricted infections [4]. As such, these donors are exposed to pathogens that can potentially be transmitted to recipients of the donor's organs. Additionally, there are newer techniques to identify many pathogens that may be transmitted from the donor to the transplant recipients [5, 6]. Finally, high-profile reports of several donor-derived infections have heightened awareness of donor-derived infections and have likely contributed to increased recognition [7–19]. In this chapter, the incidence, methods of identification and prevention, and management of unexpected donor-derived infections will be reviewed. Often, donors are expected to transmit infection (i.e., CMV donor seropositive, recipient seronegative) because of information known by the transplant team posttransplant. In most cases, such information will lead to interventions to reduce the incidence and severity of transmitted disease and is reviewed elsewhere in this text.

In the United States, the Organ Procurement and Transplantation Network (OPTN) policy sets up the framework for minimizing and tracking cases of donor-derived infection transmission. This policy includes language that defines donors at enhanced risk of disease transmission (Table 8-1); the need to obtain special informed consent before using organs from donors with known transmissible disease or risk factors for disease transmission; the need to develop local polices for screening recipients for transmitted disease posttransplant, if appropriate; and the need to report proven or potential disease transmission; policy also defines the requirements for living and deceased donor screening (http://optn.transplant.hrsa.gov/governance/policies/). The Council of Europe has also developed a similar guidance document that is updated regularly to provide similar guidance on donor screening and risk mitigation (https://www.edqm.eu/en/organ-transplantation-mission-67.html). Likewise, the European Union and national governments have established laws and directives that regulate these same functions outside the United States.

8.2 Donors at Increased Risk of Infectious Disease Transmission

Experience has demonstrated that donors with documented infections pose a risk of transmission of the infection from the donor to the recipient. While the risk of transmission varies (i.e., low risk with appropriately treated documented bacterial meningitis or *Naegleria* encephalitis, high risk with active HIV infection), the fact that there is a risk of disease transmission requires several key steps:

1. The organ procurement organization must inform the recipient center of the potentially transmissible infection.
2. The recipient center must assess if the benefit of transplantation outweighs the risk of disease transmission.
3. The recipient center must obtain special informed consent to use the organ from the donor with recognized risk of disease transmission.
4. The recipient center must develop a plan to treat the recipient, if appropriate, to prevent disease transmission and monitor the recipient for evidence of transmitted infection.

Specific details of these key steps will be discussed in greater detail later in the chapter.

Some donors do not have documented infection but instead have engaged in behaviors or have other characteristics

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TABLE 8-1. Known conditions that may be transmitted by the donor organ that must be communicated to the transplant center prior to transplantation

- Infections
  - Syndromes
    - Unknown infection of central nervous system (encephalitis, meningitis)
    - Multisystem organ failure due to overwhelming sepsis
  - Bacterial infections
    - Bacterial meningitis
    - Bacteremia
    - Pneumonia
    - Syphilis
    - Tuberculosis
  - Fungal infections
    - Endemic mycoses: blastomycosis, histoplasmosis, coccidioidomycosis
    - Cryptococcal infection
    - Fungal sepsis (e.g., *Candidemia*)
  - Parasitic infections
    - *Trypanosoma cruzi*
    - *Leishmania*
    - *Naegleria fowleri*
    - Strongyloides
    - Toxoplasmosis
  - Prion disease, including Creutzfeldt–Jakob disease
  - Viral infections
    - Active hepatitis A, B, or C
    - Herpes simplex encephalitis or documented viremia
    - Human immunodeficiency virus/AIDS
    - HTLV-I
    - History of JC virus infection (progressive multifocal leukoencephalopathy)
    - West Nile virus infection
    - Cryptococcal infection of any site
    - Rabies
    - SARS, MERS-CoV, influenza
    - Malignancies
  - Any known or history of malignancies
  - Melanoma, Merkel cell, and Kaposi's sarcoma
  - Hodgkin's disease and non-Hodgkin's lymphoma
  - Multiple myeloma
  - Leukemia
  - Aplastic anemia agranulocytosis
- Inborn errors of metabolism
- Drug or food allergies

TABLE 8-2. Risk factors for recent HIV, HBV, or HCV infection/increased risk donor criteria [20]

Criteria	Characteristics
Behavior and history	1. People who have had sex with a person known or suspected to have HIV, HBV, or HCV infection in the preceding 12 months
	2. Men who have had sex with men (MSM) in the preceding 12 months
	3. Women who have had sex with a man with a history of MSM behavior in the preceding 12 months
	4. People who have had sex in exchange for money or drugs in the preceding 12 months
	5. People who have had sex with a person who had sex in exchange for money or drugs in the preceding 12 months
	6. People who have had sex with a person who injected drugs by intravenous, intramuscular, or subcutaneous route for nonmedical reasons in the preceding 12 months
	7. People who have injected drugs by intravenous, intramuscular, or subcutaneous route for nonmedical reasons in the preceding 12 months
	8. People who have been in lockup, jail, prison, or a juvenile correctional facility for more than 72 consecutive hours in the preceding 12 months
	9. People who have been newly diagnosed with, or have been treated for, syphilis, gonorrhea, *Chlamydia*, or genital ulcers in the preceding 12 months
Pediatric only criteria	1. A child $\leq$ 18 months of age and born to a mother known to be infected with, or at increased risk for, HIV, HBV, or HCV infection
	2. A child who has been breastfed within the preceding 12 months and the mother is known to be infected with, or at increased risk for, HIV infection
HCV risk only	1. People who have been on hemodialysis in the preceding 12 months
Laboratory and other	1. Screening specimens are hemodiluted
	2. Donor medical/behavioral history is unavailable

that place the donor at increased risk of infection with pathogens, such as HIV, hepatitis B, and hepatitis C, that can, in turn, be transmitted to the recipient. These donors have been defined by the OPTN and US Public Health Services (PHS) as donors at increased risk of disease transmission, termed increased risk donors. The PHS updated their guidance related to increased risk donors in 2013, and this guidance has been adopted as the standard for most transplant systems globally [20]. This updated guideline refined prior guidance taking into account current knowledge of the epidemiology of HIV, HBV, and HCV in the community and the limitations of our contemporary screening practices (Table 8-2). The guideline focuses on three key core recommendations:

1. Screening:
   (a) There is no single standardized and validated tool for collecting the donor's medical and social history, although many US OPOs are utilizing the Uniform Donor Risk Assessment Interview Tool (http://www.

aatb.org/DRAI-Documents). Although living and deceased donors are considered to be equal risk in the guidelines, living donors are able to provide their own history, while histories from deceased donors are obtained from friends and relatives. These individuals may not know the fine details of the donor's social situation (e.g., the mother of a college student who does not live at home). As such, the guideline recognizes these limitations and places donors with incomplete donor histories in the increased risk category as risks may be present but unrecognized.

(b) The 2013 guidelines newly recommend that all donors be screened with serology and nucleic acid testing (NAT) for hepatitis C, regardless of risk factors, and that all increased risk donors be screened with HIV NAT in addition to routine serology. At the present time, only serology is mandated for hepatitis B screening, although this serologic assessment includes hepatitis B surface antigen (HBsAg) which allows for direct detection of the virus. The addition of NAT screening to serology will allow increased detection of acute infections as NAT decreases the length of time between initial infection and the ability of the test to detect the infection, referred to as the window period (Figure 8-1).

(c) The guideline also recognizes that living donors may continue to engage in behaviors that place them at increased risk of disease transmission between screening and donation. As such, the guidelines recommend that living donors are screened as close to the donation procedure as possible, not to exceed 28 days. The feasibility of this recommendation has been demonstrated clinically [21].

2. Consenting: Any patient who is to receive an organ from a patient with risk factors should understand the risk and agree to receive the organ based on that risk assessment. A specialized informed consent for increased risk donor organ use is mandated by policy.

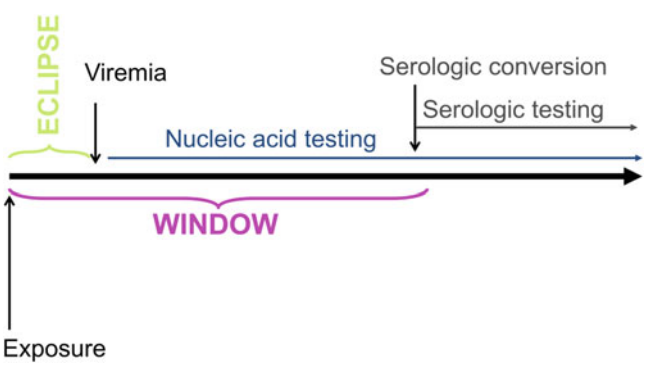

FIGURE 8-1. Interval between infection, detection of virus, and detection of antibody response to infections.

3. Follow-up testing: Perhaps one of the most important recommendations of the PHS guidelines is the need to do posttransplant testing of recipients that received increased risk donor organs to ensure that a disease transmission has not occurred. Early testing may affect outcomes if a transmission is identified, as available effective therapy can be introduced sooner after transmission. Recommendations include HIV NAT (or combined antibody–antigen assay), HCV NAT, HBV NAT, and HBsAg at 1–3 months posttransplant and HBV serology at 12 months posttransplant (including hepatitis B surface antibody, hepatitis B core antibody, and either HBV NAT or HBsAg) [20]. However, data as of 2011 show that posttransplant testing is not reliably performed [22]. Using assays that directly detect the virus in the posttransplant period is critical, and serology may not be reliable because recipients frequently fail to seroconvert due to transmitted infections. In all cases of HCV transmission identified in the United States, for example, all recipients have been seronegative but NAT positive even when tested nearly 1 year posttransplant [23, 24].

These guidelines are helpful in defining donors at increased risk of disease transmission by identifying donors with higher likelihood of HIV, HBV, and HCV infections. Review of existing data clearly demonstrates that the risk of HIV and HCV infection varies significantly by risk behavior (Table 8-3). Given the enhanced risk, it is important to realize that patients may acquire infection in the NAT window, the period of time between infection and when NAT can detect infection (see below). As such, while NAT decreases the risk of disease transmission, residual risk remains and has clearly been demonstrated by three recent transmissions of HCV from donors engaged in nonmedical drug use prior to death with negative donor NAT testing [23].

8.3 Incidence of Unexpected Donor-Derived Infections

To date, there are limited prospectively collected data on the incidence of donor-derived infections. Prior to the establishment of the OPTN/United Network for Organ Sharing (UNOS) Ad Hoc Disease Transmission Advisory Committee (DTAC) in 2005, there were no systems in place to prospectively collect data to estimate the incidence of donor-derived infections; data was only available from published case reports. Underreporting to DTAC was common initially, but recent data show a substantial increase in the numbers of reports (Table 8-4 and Figure 8-2) [24, 25]. In the era of current screening, the following unexpected transmissions have been reported: numerous bacterial species (including gram-positive cocci and gram-negative rods), *Ehrlichia chaffeensis*, legionella, syphilis, *M. tuberculosis, Candida* spp.,

Table 8-3. Residual risk of undiagnosed human immunodeficiency virus (HIV) and hepatitis C virus (HCV) infection per 10,000 donors at increased risk of infection [60, 61]

	HIV		HCV	
Risk factor	Serology alone	Serology + NAT	Serology alone	Serology + NAT
Men who have sex with men	8.3	3.4	36.0	3.8
Nonmedical intravenous, intramuscular, or subcutaneous drug use	12.9	5.3	350.0	37.8
Hemophilia	0.05	0.02	0.46	0.05
Persons who have had sex in exchange for money or drugs	2.9	1.2	107.8	11.5
Partners with any of the above risk factors	2.7	1.1	126.2	13.5
Individuals who have been exposed to blood or blood products from someone with HIV or HCV	1.3	0.5	22.0	2.3
Incarceration	1.5	0.6	68.6	7.3

Residual risk is the rate of undetected infection depending on risk factor and testing strategy.

Table 8-4. Summary of reported cases to the OPTN/UNOS Ad Hoc Diseases Transmission Advisory Committee, 2005–2014

Disease type	# of donor reports	# of recipients with confirmed transmission	# of donor-derived disease-attributed recipient deaths
Malignancies	374	79	28
Viruses	366	80	18
Bacteria	313	55	13
Mycobacteria	95	11	3
Fungi	165	45	15
Parasites	62	41	14
Other diseases	73	6	1
Total	1448	317	92

histoplasmosis, zygomycosis, *Aspergillus* spp., scedosporiosis, coccidioidomycosis, cytomegalovirus, HIV, HBV, HCV, adenovirus, coxsackievirus, human T-lymphotropic virus, lymphocytic choriomeningitis virus and a related arenavirus, West Nile virus (WNV), rabies, schistosomiasis, strongyloides, *Trypanosoma cruzi*, microsporidiosis, and *Balamuthia* spp. [7–9, 11–13, 15–19, 23–38]. From the available data from the US and French systems, donor-derived disease is transmitted in less than 1% of transplants, with approximately 0.03% of recipients dying from the transmitted disease [24, 25].

There are several key points that can be learned from the data collected to date. While confirmed bacterial transmissions are not the most commonly reported transmission, they likely represent the most common form of disease transmission. Most of the confirmed cases of bacteria transmissions involve highly resistant gram-positive and gram-negative infections. From a series of historical studies, 5–9% of abdominal organs and up to 63% of thoracic organs appear to be contaminated with bacterial pathogens at the time of procurement [25, 39–42]. Use of perioperative antibiotics reduces the risk of disease transmission, although under-recognition and underreporting of bacterial transmissions are likely. Given the high rate of contamination and the increasing prevalence of highly resistant bacteria in hospitals globally, bacterial transmissions will increase over time [25, 39–42]. As such, diligence is important among transplant teams.

Although there have been attempts to estimate the risk of donor-derived infections, none can be considered accurate as there is no formal screening process to identify potential transmissions, and the issues of under-recognition and underreporting of transmissions remain. It is critical, and required by current UNOS Policy, that everyone caring for transplant recipients considers the potential of donor origin in all infections, particularly early posttransplant, and has a plan in place to report this concern to the local OPO and to UNOS [43]. Organ vigilance systems, similar to the OPTN/UNOS Patient Safety System, contribute to more rapid communication. Efficient and timely communication is associated with a lower rate of recipient adverse events, including death [44]. As such, regions without organ vigilance systems should establish formal systems, as has recently been required by the EU directive, to improve patient outcomes and potentially improve the safety of the transplant system.

8.4 Prevention of Infectious Transmissions

The mainstay of infection prevention in organ transplantation is the use of donor and recipient screening. Despite best efforts to screen for potential infections and in a timely manner, the transplanting physicians and organ recipients must understand there always remains a risk for infectious transmission. The goals of screening donors and recipients prior to transplant are to identify conditions that disqualify the donor or recipient from the transplant, to identify and treat active infections pre-transplant, and to allow for risk mitigation strategies to minimize posttransplant infections. Screening occurs in many forms including acquisition of a careful history, detailed physical examination, detection of latent or unknown active infections by laboratory testing, examination

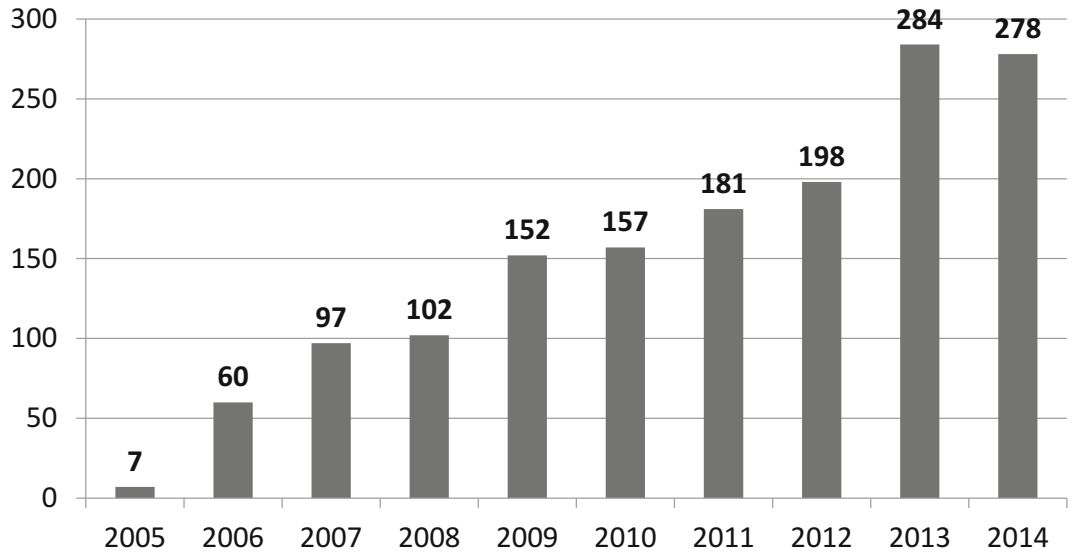

FIGURE 8-2. Potential donor-derived disease transmission reports to the OPTN/UNOS Ad Hoc Disease Transmission Advisory Committee.

TABLE 8-5. Infectious disease screening tests recommended for all organ donors

Required by OPTN policy	HIV 1/2 antibody *or* HIV antigen/antibody combination test[a]
	Cytomegalovirus (CMV) antibody[b]
	Hepatitis B surface antigen (HBsAg)[a]
	Hepatitis B core antibody (HBcAb)[a]
	Hepatitis C antibody[a]
	Hepatitis C NAT
	Syphilis test[b]
	Epstein–Barr virus (EBV) antibody[b]
	Blood and urine cultures
	Sputum gram stain (lung transplant donors only)
	Toxoplasma antibody test result or appropriate donor sample to be tested at transplant hospital (heart donors only)
Recommended donor screening	Coccidioidomycosis serology[c]
	Strongyloides[c]
	TB screening (PPD or interferon-γ release assay)[c]
	Trypanosoma cruzi serology[c]
	West Nile virus testing[c]
	Serologies to help guide pre-transplant vaccination: tetanus, diphtheria, measles, mumps, and *S. pneumoniae*

[a]Must be an FDA-approved, cleared, or licensed donor screening tests.
[b]Can be donor screening *or* diagnostic test.
[c]See text for detailed recommendations on testing situations.

TABLE 8-6. Infectious disease screening tests recommended for all organ recipients

Recommended recipient screening tests for all donors	HIV 1/2 serology[a]
	Anti-cytomegalovirus antibody
	Hepatitis B surface antigen (HBsAg)
	Hepatitis B surface antibody (HBsAb)
	Hepatitis B core antibody (HBcAb)
	Hepatitis C antibody
	VDRL or RPR
	EBV serology
	Varicella-zoster virus antibody
	Toxoplasmosis antibody (for heart recipients)
	TB screening (PPD or interferon-γ release assay)
Recommended recipient screening tests for selected donors	HSV 1/2 IgG antibody
	Trypanosoma cruzi serology
	Strongyloides serology
	Coccidioidomycosis serology

and pathologic evaluation of the organ at the time of procurement and implantation, and posttransplant monitoring of recipients. There are policies that have been developed by the OPTN that mandate which screening tests must be done in all donors and recipients (see Tables 8-5 and 8-6). A number of guidelines and consensus conferences have further refined the screening of donors and recipients [20, 45–55].

8.4.1 Donor Screening Methods

While donors undergo a range of screening, including review of the donors' medical and social history and physical examination of the donor and their organs, most attention is paid to tests that are performed on the donors blood to risk stratify the donor. Mandated screening of blood has traditionally focused on detection of antibodies or antigens present in donors, typically using enzyme-linked immunosorbent assays (ELISA) for most infectious disease screening. As of 2011, molecular screening methods using NAT for HIV, HBV, and HCV screening had been implemented by most US organ procurement organizations [56]. In 2014, OPTN policy was updated to mandate HCV NAT for all deceased donor screening and HIV NAT for screening of increased risk organ donors [20, 57].

The indirect ELISA is used to detect antibodies (i.e., HBcAb) while the sandwich ELISA is used to detect antigens (i.e., HBsAg). In the indirect ELISA (http://www.paho.org/hq/index.php?option=com_topics&view=article&id=10&Itemid=40743), a known antigen is fixed to the bottom of a plastic surface, usually a multi-well plate. Serum is then added and if antibodies that react to the antigen are present, they bind to the antigen. The plates are then washed and a detection antibody (usually an anti-IgG or anti-IgM antibody) that is conjugated to a substrate-specific enzyme is applied to each well. After washing, a substrate is then applied and is converted by the enzyme conjugated to the detector antibody, typically resulting in a colorimetric change. The intensity of this change provides a semiquantitative measurement of the presence of the antigen-specific antibody. To detect an antigen, a sandwich ELISA is used in which a capture antibody (one which is specific for the antigen of interest) is bound to the plates. The patient's serum is applied and if antigen is present, it is bound by the capture assay. The plates are then washed and antigen-specific antibodies are applied—creating an antibody–antigen–antibody sandwich. Detection antibodies and substrate are added as above and the resultant colorimetric change is read. Both of these methods have relatively rapid turnaround times, are not subject to a significant risk of contamination, and can usually be done by either an automatic machine or with minimal technical skills.

As discussed below, there are clear challenges to these serological assays. To detect infection earlier, before antibodies have been created, NAT is used for screening of certain infections. NAT refers to a wide range of polymerase change reaction (PCR), transcription amplification testing (TMA), and branched DNA tests. PCR is the most widely used test in which primers that code for complimentary regions of a pathogen-specific gene of interest are selected. For RNA viruses, the RNA is first reverse transcribed to create a complementary DNA (cDNA) based on the RNA template. At this point, PCR for both RNA and DNA viruses are the same. The source nucleic acids are then mixed with the selected primers, DNA polymerase, deoxynucleoside triphosphates, and buffer materials. An initialization step activates the polymerase, and then a denaturation step melts the DNA into two single strands so that the primers may anneal. Extension or elongation then occurs and repeated cycles proceed to amplify the presence of the gene of interest. The presence of DNA can be detected in one of several ways. In real-time PCR, fluorescent dyes are used to intercalate into the double-stranded DNA to quantitatively detect the target DNA. NAT is challenged by longer turnaround time than serologic tests, greater technical expertise to perform the test, and risk of contamination that may result in false-positive test results, especially in low-volume laboratories.

8.4.2 Challenges to Current Screening Techniques

Once an individual is infected with a viral infection, there is typically local replication of the virus with subsequent viremia (see Figure 8-1) [58]. The period of time between initial infection and detectable viremia is referred to as the eclipse period. Once viremia is present, the immune system can recognize the virus and produce neutralizing antibodies to components of the virus [58]. The period between initial infection and the first detection of these antibodies is referred to as the window period [59]. Transmission of both HIV and HCV from donor to recipient has been reported to UNOS during both the eclipse and serologic window periods [17, 23, 24]. The window period differs for each virus and has been shortened over time with improved serologic tests that are able to detect antibodies earlier after initial infection (Table 8-7) [60, 61].

Unfortunately, even once antibodies are formed, it may be challenging to detect these antibodies. First, some donors require transfusion of blood and blood products or receive large volumes of fluids to replete their intravascular compartments. This may dilute the concentration of the antibodies, antigens, or viral particles to below the limit of detection; this process is referred to as hemodilution [62]. There are a number of ways in which hemodilution can be assessed, and no one method is currently considered the gold standard; a simple guideline is that testing may be less reliable if the donor has received greater than 2 L of blood or crystalloid within 48 h of blood sampling or greater than 1 L of crystalloid within 1 h of sampling in adults; recommendations are more strict for pediatric donors [62, 63]. Second, some donors may lose their serologic response to certain infections over time, particularly if they are immunosuppressed. Thirdly, in infant donors, serologic testing may detect the presence of maternal antibodies without active infection of the child [64].

8.4.3 Donor Types

The type of donor affects testing as well. In general, there are two types of donors: living and deceased donors. The major differences between these donor types are the potential quality of the donor history and the time frame during which

Table 8-7. Interval between initial infection and detection of infection by current molecular and serologic testing methods [136, 137]

Pathogen	First detection by NAT (days)	First detection by serology (days)
HIV	7	22
HBV	20	59
HCV	7	70

donor screening may take place. In the case of living donation, the actual donor is interviewed, which may allow for collection of a more accurate medical and behavioral history. The quality of the medical and behavioral history is more challenging in deceased donation in which the histories are obtained from friends and family members who may not know all of the medical or behavioral details—especially if there was limited contact between the donor and the historian. Often, the clinical circumstances leading to the death of the deceased donor may contribute to this limitation—often donors are found down and accurate history about the preceding events may be limited [13, 15]. Likewise, donors may have undergone extensive resuscitation and/or a prolonged hospitalization that will affect donor screening and infectious risk—secondary to hemodilution as discussed above or by introduction of infection at the time of transfusion [7, 11]. There may be a significant time frame between initial screening and organ donation in the case of a living donor. As such, consideration of repeat testing closer to the time of the transplantation should be considered, and current policy recommends that HIV, HBV, and HCV screening of all living donors be performed within 28 days of the transplant [65]. When time allows, donors may be treated for potentially transmissible infections, such as latent TB, prior to the transplant, decreasing the transmission risk. Lastly, the period of time between initial evaluation and transplantation allows one to screen living donors with risk factors for contracting blood-borne viral infections, such as HIV, HBV, and HCV. In increased risk living donors, as defined by the 2013 Public Health Services Guidelines [20], counseling to abstain from the increased risk behaviors and repeat NAT and serologic testing over a period of time similar to the window period for the virus of interest should be considered to minimize the risk of an occult transmission [20].

8.4.4 Universal Donor Screening

As previously stated, screening of the potential organ donor is critical to identify pathogens that can be transmitted to a recipient. Current OPTN policy requires that screening be done for certain pathogens (see Table 8-5) and that enhanced cultures be obtained in the setting of hospitalization of ≥72 h [66]. Current policy requires only sputum gram stain and description of sputum for lung donors and does not require collection of BAL specimens for cultures (although only bacterial cultures are frequently done by some OPOs, viral, fungal, and mycobacterial cultures are typically not obtained) [66]. As of December 2014, NAT is required for HCV screening of all donors and for HIV screening of increased risk donors only. Positive NAT results suggest active viremia; as such appropriate measures need to be in place for appropriate consent and prophylaxis of and follow-up of recipients for transmission events if viremic donor organs are to be used. Negative NAT does not rule out infection, although it may suggest a lower risk of transmission.

8.4.5 Donor Screening for Endemic Infections

Over time, new pathogens have become significantly prevalent and recognized as having transmission potential via organ transplantation. Screening for these types of pathogens should be considered based on local prevalence of the disease. Endemic pathogens that have increasingly been recognized to result in disease transmission and likely should be screened for in donors from endemic regions include: Chagas disease (*Trypanosoma cruzi*), coccidioidomycosis, strongyloides, and WNV.

8.4.5.1 Chagas Disease (Trypanosoma cruzi)

Chagas disease is caused by the parasite *Trypanosoma cruzi* and is endemic to regions of Central and South America [67, 68]. Since testing for Chagas is required for all blood donors, seroprevalence in the United States is known, and significant geographic variability is recognized [53]. Available US guidelines recommend targeted *T. cruzi* screening of potential donors born in Mexico, Central America, and South America. Given the high rate of false-positive results, donors with initially reactive results should have confirmation with a second test. These guidelines suggest that programs can consider transplantation of kidneys and livers from *T. cruzi*-infected donors with informed consent from recipients but do not recommend the use of heart transplantation from infected donors [53]. Recipients of *T. cruzi*-infected donors should be monitored posttransplant with PCR-based screening with institution of antitrypanosomal treatment if recipient infection is detected [53].

8.4.5.2 Coccidioidomycosis

While there is a low but true risk of disease transmission with all endemic fungal infections, the risk appears highest for coccidioidomycosis. This is likely because of the risk of donor transmission as well as recipient reactivation of disease. Coccidioidomycosis is endemic to the Sonoran desert in the Southwest of the United States and Northwest of Mexico in addition to Central and South America. Donors and recipients from endemic regions should be screened for seropositivity by enzyme immunoassay (EIA), complement fixation (CF), or immunodiffusion (ID). If the donor or recipient is seropositive, prophylaxis with fluconazole, typically 400 mg initially (3–12 months) followed by 200 mg daily, is recommended [69].

8.4.5.3 Strongyloides

There have been an increasing number of donor-derived strongyloides transmissions in the United States, likely secondary to a large pool of potential donors with latent infection and increasing use of steroids for donor maintenance

[27]. Strongyloides is endemic in tropical or subtropical regions of the world, where seropositivity may exceed 80% in some locations. Historically, high rates of strongyloides (~3.8%) have been documented in Appalachia and the southeastern United States. Current guidelines recommend routine screening of donors from endemic regions for strongyloides IgG. Living donors should be treated with ivermectin 200 μg/kg daily on two separate days prior to donation, whereas recipients of deceased donors with positive strongyloides antibodies should receive ivermectin posttransplant [54, 70].

8.4.5.4 West Nile Virus

All US blood donors are screened for WNV. The presence of antibodies to WNV does not predict risk of infection, as they are present in those with prior WNV infection or related flaviviruses. As such, detection of virus in the blood by molecular testing predicts increased risk of transmission, although there have transmissions with negative NAT [7, 11, 71]. A 2008 survey revealed that 11/58 OPOs were currently testing donors for WNV by PCR, typically performing testing in seasons when virus would be predicted in the donor service area [72]. Universal testing may be associated with loss of organs and net loss of life in transplant candidates [73]. A more effective screen is to avoid the use of donors with unexplained encephalitis or unexplained mental status change, but unfortunately donors may be completely asymptomatic and carry the infection [7, 11, 68]. Since transmission of other neuropathogens and malignancies has been associated with patients with unexplained encephalitis, avoidance of these donors is prudent in general [13, 15, 16, 31, 74, 75]. If donor testing is utilized, it should be restricted to NAT of the donor blood during periods of time when there are WNV cases in the region from which the donor resided [54]. Use of donor WNV serology or testing of urine for WNV is not recommended at this time.

8.4.6 Recipient Screening

Just as donor screening is critical to minimize the risk of posttransplant infectious complications, recipient screening may contribute to prevention of donor-derived disease transmission (Table 8-6). This is particularly important for CMV and, in the case of potential heart recipients, toxoplasmosis, where the risk of disease and prophylactic plans are frequently determined by donor and recipient serostatus. In addition, all potential recipients should be screened for tuberculosis using medical history (to assess for potential exposure, prior testing, and prior treatment), radiologic examination (baseline chest radiograph), and testing for latent tuberculosis using either the PPD, with a 5 mm cutoff for positivity, or a TB-specific interferon-γ release assay (such as QuantiFERON-TB Gold, Cellestis Inc., Victoria, Australia) [51, 76, 77]. All potential recipients should also be screened either clinically or serologically for a history of exposure to varicella-zoster virus to allow pre-transplant vaccination of unexposed candidates [51, 78].

Recipients may have been exposed to pathogens with regional endemicity. Careful travel and residence history should be obtained from all potential recipients to determine if specialized testing is indicated. Chagas disease can reactivate in asymptomatic, latently affected recipients [67]. Since Chagas is endemic throughout much of Mexico and Central and South America, consideration of screening potential candidates from affected countries should be considered. Since the approved serologic tests lack specificity, confirmatory testing is recommended; patients with confirmed positive serologic screening should be evaluated by an expert in Chagas disease before proceeding for transplantation [53, 67]. Strongyloides is a parasitic infection that is endemic to tropical and subtropical regions of the world. Infection may remain latent after initial infection with risk of potentially lethal hyperinfection in immunosuppressed patients, particularly those who receive steroids [79]. Reactivation with associated mortality has been well described in the setting of solid organ transplantation [79, 80]. As a result, serology for strongyloides and/or testing of stool for ova and parasites should be considered in all at-risk transplant candidates [80]. Lastly, some centers test patients who have lived in areas with high endemicity for *Coccidioides immitis* for serologic exposure to the fungus [81]. There is no role in testing recipients by serology for histoplasmosis or blastomycosis [82].

Certain transplant candidates may have underlying organ diseases that predispose to pathogens that warrant special screening—this is especially true among lung transplant candidates. Patients with a history of cystic fibrosis may be colonized with pathogens that are highly resistant to usual antibiotics; as such, regular screening cultures from BAL and nasal washes may allow the tailoring of specific perioperative antibiotic regimens to minimize the risk of posttransplant infections with these resistant pathogens [83, 84].

8.5 Management

8.5.1 Vaccination

Recipient serology should be a strong driver of pre-transplant vaccination. Detailed recommendations about transplant candidate and recipient vaccination are made elsewhere (see Chap. 48). Patients who do not have evidence of hepatitis B immunity should receive three doses of HBV vaccine unless contraindicated. Although the traditional regimen of vaccination at months 0, 1, and 6 is used by most centers, there is evidence that an accelerated regimen using double doses of vaccine (at days 0, 7, and 21 or weeks 0, 2, 4, and 6 or months 0, 1, and 2) may provide similar efficacy in a shorter period of time [85–87]. Serologic evidence of protection, as

demonstrated by HBs antibody seropositivity, would potentially allow the use of a core-positive alone or HBV-infected organ in a protected recipient. Likewise, patients without prior exposure to varicella-zoster virus are at increased risk of severe infection if exposed posttransplant. Nonimmune transplant candidates should be vaccinated against varicella unless they have a contraindication to vaccination. Lastly, although there are not a lot of data regarding measles and mumps posttransplant, given recent outbreaks of these diseases in the United States and abroad, measles and mumps immunity should be ensured prior to transplantation, especially in candidates born after the vaccine era (after 1963).

8.5.2 Recipients of Organs from Increased Risk Donors

As of 2015, 19.5% of all organ donors in the United States are at increased risk of having undetected HIV, HBV, and/or HCV (increased risk donors) [88]. The 2013 PHS guidelines suggest that "even though attempts should be made to ensure the highest level of safety, organ donor and recipient selection practices and policies should not be restrictive, considering the clinical need… informed decision-making is an important part of this process for transplant clinicians and their patients" [20]. Data suggest that there may be net benefit to using increased risk donor organs, especially in patients on hemodialysis who have a risk for acquiring these infections already, particularly when further screened using NAT [89]. Per current OPTN policy, "if additional donor disease or malignancy transmission risk is identified pre-transplant, the transplant program must … explain the risks and obtain informed consent from the potential transplant recipient … before transplant, document this consent in the potential recipient's medical record and follow any recipient of the deceased or living donor organs for the development of potential donor-derived disease after transplantation" [43]. Policy also states that "if a donor is found to have an increased risk for transmitting blood borne pathogens, the transplant program must offer recipients of the donor organs…., additional post-transplant testing for HIV, hepatitis C, and hepatitis B as appropriate … [and] every transplant hospital must develop and implement a written protocol for post-transplant testing … [as well as] treatment of or prophylaxis for the transmissible disease, when available" [43]. Policy also requires that the host OPO maintain "blood specimens appropriate for serologic and … NAT, as available, for each deceased donor for at least 10 years after the date of organ transplant, and ensuring these samples are available for retrospective testing" [66]. The 2013 PHS guidelines outline how recipients of increased risk donor organs should be followed and recommend that baseline serology be drawn immediately pre-transplant and the following tests at 1–3 months posttransplant: HIV NAT (or combined antibody–antigen assay), HBV NAT and HBsAg, and HCV NAT [20]. Additionally, the following tests are recommended at 12 months posttransplant: HBsAb, HBcAb, and either HBV NAT or HBsAg [20].

Transmission without seroconversion, especially in the case of HCV, has occurred in the majority of the donor-derived transmissions to date, and therefore, posttransplant testing of recipients of increased risk donor organs must use both serologic and molecular methods [17, 20, 23]. Any documented disease transmission must be reported to the local OPO and to UNOS and should warrant further evaluation as well as referral for management of the transmitted infection [43].

8.5.3 Recognition and Management of Potential Donor-Derived Infection Transmissions

There are currently a number of limitations to the recognition of potential donor-derived transmission events. Often, a single donor provides organs to recipients at multiple different centers or recipients that are cared for by different management teams at the same center. As a result, multiple recipients may present with similar clinical illnesses, but the clustering of this illness goes unrecognized [16]. Likewise, pathogens that are commonly recognized as causing nosocomial infections may not be recognized as a potential donor-derived infection [41, 42]. Finally, onset of disease may be of variable severity in individuals and may present with variable onset posttransplant, further challenging the recognition of donor-derived transmission [11, 15, 90]. To overcome these challenges, it is critical that all transplant centers:

1. Maintain a high level of suspicion for donor-derived infection in all early infections or unexplained clinical illnesses. Any early infection or unexplained clinical illness should lead to an inquiry as to the clinical status of other recipients. This is most easily done through contact with the local OPO. This is especially true for patients with unexplained neurological or severe illness within the first 30–60 days posttransplant.
2. Develop a plan for reporting concern for a potential donor-derived infection transmission. This plan should include a local contact at the transplant center and at the OPO when there is concern for a potential donor-derived infection transmission. Likewise, it is important to consider the transplant center's risk management policies to determine if others within your institution need to be alerted. This plan should also include whom the OPO should contact at your center if there are questions about the status of recipients based on queries generated from other centers. Some have found appointing a specific surgeon and transplant infectious disease consultant as the early points of contact facilitate the clear transmission of data.

3. Provide timely feedback about your patients when information is requested by the OPO.
4. Seek outside expertise, through your local health department, UNOS's Disease Transmission Advisory Committee, and the Center for Disease Control's Office of Blood, Organ, and other Tissue Safety. These groups can advise on optimal testing, help collect appropriate specimens, and may provide insight into similar cases that allow the local center to make more informed treatment decisions.

8.5.4 Management of Recipients of Organs from Infected Donors

As previously discussed, all organ donors are currently screened with serology for HIV 1/2, CMV, EBV, HBV, and HCV and also with HCV NAT as currently required by OPTN policy [66]. Although the recent passing of the HIV Organ Policy Equity Act allows for research to be performed in using organs from donors that are HIV-1/2 infected, the use of these organs for transplant is not yet allowed outside of a research protocol in the United States [66, 91].

Donors seropositive for CMV and EBV are universally used, although donor and recipient serologic status may determine monitoring and prophylaxis plans. The risk of developing CMV viremia and diseases is greatest in seronegative recipients of seropositive organs as described later in this text [92, 93]. Universal prophylaxis and preemptive therapy can reduce the risk of viremia and disease in at-risk patients [92, 93]. Development of posttransplant lymphoproliferative disorder (PTLD) has been described, particularly in young EBV seronegative recipients of EBV seropositive donors [94–96]. Careful monitoring and intervention, as described later in this text, may reduce the risk of PTLD in this setting [94–96].

Current mandated donor screening for hepatitis B includes detection of HBV surface antigen (HBsAg) and core antibody (HBcAb). HBsAg is a marker of active viremia while HBcAb is a marker of exposure to hepatitis B virus. Since HBV vaccine only contains hepatitis B surface antigen, vaccinated donors should only have HBV surface antibody (HBsAb), but not HBcAb. If HBsAg is measured early after vaccination, it can be detected in the blood [97, 98]. The risk of transmission of HBV to a nonimmune recipient is high in HBsAg-positive donors [99]; use of these organs is discussed further in the chapter on Hepatitis B. Although HBsAg-positive donors may have been deferred in the past, advances in the use of hepatitis B immune globulin and anti-HBV antivirals now allow the selective use of these organs [99]. Donors that have isolated HBcAb positivity may represent latently infected individuals or a false-positive result. The risk of transmission of HBV in liver transplant recipients is higher than in non-liver recipients [100]. The risk of transmission of HBV from a donor with and isolated HBcAb is estimated to be less than 5% for non-liver recipients [55, 101, 102]. Additional testing of these donors with HBcore IgM and HBV DNA NAT would further stratify the risk of transmission, with the highest risk in the IgM+ and NAT+ donor [55]. Some centers use these organs, particularly in patients who have been vaccinated against hepatitis B [55]. When used in nonimmune patients, posttransplant vaccination is often combined with the use of either anti-HBV antiviral prophylaxis or hepatitis B immune globulin infusions [55]. All recipients, especially liver recipients, of HBsAg+ or HBcAb+ donors should be monitored closely for the presence of active viral replication with expansion of therapy based on these results [55, 103, 104].

Detection of HCV antibodies suggests prior infection with hepatitis C, but about 15–20% of those infected with HCV will clear the virus, and the HCV serology test has a relatively high known false-positive rate [105, 106]. Therefore, the use of HCV NAT in donor screening allows for differentiation between prior HCV infection and active infection with viremia. Use of HCV-positive donors for HCV-negative recipients is currently considered only in life-threatening situations; however, HCV-positive donor organs should be considered in HCV-infected recipients [106–109]. Small, likely clinically insignificant, decreases have been found in transplant graft and patient survival when HCV+ organs are used for HCV-infected recipients, but these are offset by the concomitant decrease in transplant waiting list time [108]. Mortality appears to be higher among heart recipients, so the use of HCV seropositive donors is less frequently considered [110–112]. One concern relates to infecting a recipient with an additional HCV genotype that may be less responsive to antiviral therapy, since genotype results are typically not known at the time of transplant. With the advent of new and evolving data regarding direct-acting antivirals and interferon-free HCV regimens, the options to treat HCV before and after transplant are continuously changing.

It should be noted that the only required bacterial screening in organ donors is blood and urine cultures of donors and screening for syphilis. OPOs are required to determine if additional culture-based testing has been conducted on the donors prior to procurement. Unfortunately, there are current challenges to this—once the donor is declared deceased, they are often "discharged" from the hospital and then readmitted under a new account under the care of the OPO until procurement. When cultures come up positive, the laboratory may not realize that the deceased patient has become a donor and that they have to inform the OPOs of the donor result. Some hospitals may stop working up cultures on "deceased" patients so that no further results are obtained. Lastly, follow-up cultures of bacteremic donors may not be available at the time of procurement.

In general, donors with positive blood cultures may be used if they have received an appropriate antimicrobial and have had a clinical response to therapy; often a complete course of therapy is given to the recipient posttransplant.

Transmission of particularly virulent organisms, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, has been described [37, 40, 113]. Donor bacteremia or candidemia mandates treatment of all recipients with a minimum of 14 days of appropriate, active systemic therapy, and experts recommend 4 weeks of recipient therapy when receiving organs from donors bacteremic with *Staphylococcus aureus* [114]. Non-bacteremic localized infections from other sites only require treatment if transmission in the transmitted organ is plausible (i.e., positive urinary cultures require therapy in kidney recipients; sputum cultures require therapy for lung recipients but not other recipients unless bacteremic; etc.).

In patients with proven bacterial meningitis, even with bacteremia, organs can be safely used as long as the patient has received at least 24 h of appropriate antibiotics and antibiotics are continued in the recipient [115–118]. Donor bacterial colonization of lung donors is common. Donor lung sample, including donor bronchoscopy at the time of lung transplantation, may allow for directed antimicrobial therapy.

All donors are tested for latent infection with syphilis per OPTN policy. A recent survey of OPOs revealed that 87% used RPR for testing and 81% of OPOs confirmed positive initial tests with a confirmatory test; a high rate (41%) of positive RPR results was negative on confirmatory testing [119]. Transmission of syphilis by organ transplantation has been rare and is not a contraindication to organ donation [120–122]. Recipients are typically treated as latent syphilis of unknown duration with 3 weekly 2.4 million unit doses of benzathine penicillin G [51, 122].

Lastly, sometimes perfusate or transport media may become contaminated with bacteria or fungi. As with other infections, this is a risk factor for systemic infection and formation of mycotic aneurysms at the site of vascular anastamoses. A full 14-day course of active antibiotic is recommended for recipients to minimize the risk of transmission [123–125].

In general, screening for *Mycobacteria tuberculosis* is not done in deceased donors, but should be performed in all living donors [51, 54, 126, 127]. Active tuberculosis in any donor is a contraindication to donation; if a deceased donor is thought to possibly have tuberculosis, their organs should not be used unless active TB infection can be definitively ruled out [126, 128–131]. Donor-transmitted TB accounts for 4% of all posttransplant cases of tuberculosis [132]. Testing for TB can be done by the PPD placed using the Mantoux method or via a TB-specific interferon-γ release assay (such as QuantiFERON-TB Gold, Cellestis Inc., Victoria, Australia) [133]. Positive testing by either method should result in careful assessment for active disease, including chest imaging and, if appropriate, sputum and/or urinary AFB cultures. Some centers will provide latent TB treatment to the recipient of organs from these donors; this decision can be individualized as transmission if not universal [77, 129, 132]. If the recipient does not receive treatment for latent TB, a note about the donor's testing should be prominent in the recipient chart to trigger aggressive evaluation with the appropriate clinical presentations (i.e., sterile pyuria, pneumonia).

Toxoplasmosis is a parasite that remains dormant predominantly in muscle tissue. As such, the risk of transmission is greatest in heart donation [68]. Routine screening of all donors for toxoplasmosis is not done at many OPOs, but policy requires that serum is procured at the time of explanting the heart to perform toxoplasmosis serology at the recipient center [66, 134]. Positive serology is not a contraindication for transplantation. In the setting of heart transplantation, donor and recipient toxoplasma serostatus may affect prophylactic and monitoring strategies [134, 135]; generally, prophylaxis is not modified in non-heart recipients of toxoplasmosis seropositive donors [135].

8.6 Conclusion

Donor-derived infections are increasingly recognized as causes of morbidity and mortality that typically present in the early posttransplant period. Careful screening of donors through history, physical examination, and serologic and molecular testing may minimize the risk of infection transmission. It is impossible to screen for all potential pathogens, and our current screening practices have clear limitations. As a result, the possibility of donor origin should be considered for all early infections and patients with atypical clinical courses. Reporting of proven or suspected donor-derived infections is currently mandated as part of OPTN policy.

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9 Transplant Infections in Developing Countries

Clarisse M. Machado

9.1 Introduction

A significant number of developing countries are located in the tropical or subtropical regions thereby having epidemiological characteristics different from developed countries with temperate climates. Along with the social and economic challenges, these countries are characterized by the occurrence of endemic infections and diseases that are absent or rare in developed countries.

The rising success rate of solid organ (SOT) and hematopoietic stem cell transplantation (HSCT) together with modern immunosuppression make transplants the first therapeutic option for many diseases affecting a considerable number of people worldwide. Thus, populous developing countries have faced a growing need of transplant procedures and struggled to develop public financing programs for SOT and HSCT to assure universal accessibility and avoid any kind of commerce [1].

Some developing countries are among the top ten countries performing the highest absolute number of kidney and liver transplants worldwide. In 2013, 5433 kidney and 1723 liver transplants were performed in Brazil, behind only the United States. Mexico and South Korea were also among the ten countries performing the highest absolute number of kidney transplants worldwide. Argentina, China, India, and South Africa, have also shown a sustained economic growth and the amplification of transplant programs is now a reality [2].

In recent years, developing countries had to adjust international transplant guidelines to their epidemiological characteristics, including the local policies and management of tropical diseases.

Table 9-1 shows the most prevalent tropical infectious diseases in developing countries. Although highly prevalent in general population in these countries, some of them have not been described in transplant recipients or do not seem to confer an increased risk in this population. Others, such as Chikungunya and Zika virus, have been recently recognized as potential threats to be confirmed in the next years.

This chapter reviews the epidemiology of viral, bacterial, and protozoan tropical diseases with greatest disease burden that have affected transplant recipients in developing countries or may represent a threat to transplant recipients living in other regions. Endemic mycosis and soil and water-associated tropical infections are reviewed in the other chapters of this book.

9.2 Viral Infections

9.2.1 Dengue

Dengue is the most rapidly spreading mosquito-borne viral disease in the world and occurs both as an endemic or epidemic disease. In the last 50 years, incidence has increased 30-fold with increasing geographic expansion to new countries and, more recently, from urban to rural areas. An estimated 50 million dengue infections occur annually and approximately 2.5 billion people live in areas where dengue viruses can be transmitted [3].

Dengue virus (DEN) is a small single-stranded RNA virus comprising four distinct serotypes (DEN1, DEN2, DEN3 and DEN4). These closely related serotypes of the dengue virus belong to the genus Flavivirus, family Flaviviridae, which are transmitted by mosquitoes of the genus Aedes, such as *Aedes aegytpi* and *Aedes albopictus*.

Infection by one serotype provides lifelong immunity against that serotype but only partial and transient protection against subsequent infection by the other three. In the immunocompetent population, there is good evidence that secondary infection increases the risk of more serious disease resulting in dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). Patients with severe dengue have elevated circulating levels of T-cell activation markers, such as IL-8, IL-10, TGF-β, and interferon-γ [4]. Therefore, a robust immunologic response is a prerequisite for the development of DHF or DSS.

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Table 9-1. Distribution of tropical infectious diseases according to region[a]

Region	Viruses	Bacteria	Parasites
North Africa	Hepatitis A, rabies	Plague, tuberculosis, typhoid, and paratyphoid fever	Leishmaniasis
Sub-Saharan Africa	Dengue, zika virus, yellow fever, chikungunya, rabies, hepatitis A, hepatitis B, poliomyelitis, other viral hemorrhagic fevers	Diphtheria, tuberculosis, plague, leprosy	Leishmaniasis, malaria, schistosomiasis, trypanosomiasis
Mexico, Central America	Dengue, hepatitis A	Leptospirosis, typhoid, and paratyphoid fever	Leishmaniasis
Latin America	Dengue, zika virus, chikungunya, yellow fever, rabies, hepatitis A, hepatitis B, measles, hantavirus, other viral hemorrhagic fevers	Tuberculosis, leprosy, leptospirosis, plague	Leishmaniasis, malaria, schistosomiasis, trypanosomiasis
South-East Asia	Dengue, Zika virus, hepatitis A, hepatitis B	Leptospirosis, plague, Tuberculosis	Filariasis, malaria, schistosomiasis
South Asia	Chikungunya, hepatitis A, hepatitis B, rabies	Leptospirosis, plague, tuberculosis	Filariasis, leishmaniasis, malaria
East Asia	Hantavirus, hepatitis A, hepatitis B	Leptospirosis, plague, tuberculosis	
Northern Asia	Hantavirus, hepatitis A, hepatitis B, rabies	Diphtheria, tuberculosis	
Middle East	Hepatitis A, hepatitis B	Tuberculosis	Leishmaniasis

[a](*) Include only diseases with widespread transmission, epidemic activity or high risk for infection in some areas.

Dengue has a wide clinical spectrum varying from asymptomatic to severe and non-severe clinical manifestations. It is recognized that the number of asymptomatic carriers is at least three times that of dengue fever (DF) cases. After the incubation period, the illness begins abruptly as a flu-like illness. Symptomatic dengue virus infections are grouped into three categories: undifferentiated fever, dengue fever (DF), and DHF. DSS is the most severe forms of DHF (severity grades III and IV). Warning signs for dengue complications are abdominal pain or tenderness, persistent vomiting, clinical fluid accumulation, mucosal bleeding, lethargy, liver enlargement >2 cm, and increase in hematocrit concurrent with rapid decrease in platelet count. However, it is important to highlight that dengue may have several clinical presentations, often with unpredictable clinical evolution and outcome. The absence of warning signs does not preclude the possibility of severe disease and lethal outcome [3].

9.2.1.1 Geographic Distribution

More than 70% of the population at risk for dengue worldwide lives in the South-East Asia Region and Western Pacific Region. In the region of the Americas, an interruption of dengue transmission was temporarily reached during the 1960s and early 1970s, as a result of the *Ae. aegypti* eradication campaign. However, vector surveillance and control measures were not sustained and there were subsequent re-infestations of the mosquito, followed by outbreaks in the Caribbean, and in Central and South America. In the last 13 years, dengue outbreaks were reported in Brazil, Honduras, El Salvador, Equator, India, Indonesia, Southwest Indian Ocean, Cape Verde, Crimean-Congo, Pakistan, and Madeira Island. Only in Brazil, three outbreaks occurred during this period, in 2002, 2008, and 2015 [5].

In the region of the Americas, 871,150 cases of dengue/severe dengue and 272 deaths were reported up to May 8th 2015 (epidemiological week 16), with a case-fatality rate of 0.03. More than 85% of those cases occurred in Brazil [6].

9.2.1.2 Transmission in Transplant Recipients

Several countries in the aforementioned regions have active transplant programs. In the last 5 years, an increasing number of dengue cases have been described in transplant recipients, as a consequence of the growing incidence of dengue in several countries [3]. So far, more than 150 dengue cases have been reported [7–18]. The numbers may be even higher since most of the cases are mild and presenting as a flu-like syndrome. Thus, many cases have certainly been underdiagnosed in transplant population from endemic areas. Dengue can be transmitted by mosquito bite, blood transfusion, or by the graft.

Mosquito bite. The most frequent mode of dengue transmission in transplant recipients is through mosquito bite. According to the published cases, more than 95% of the transplant recipients were living in or have traveled to an endemic area and acquired dengue by vector transmission [8–19].

Graft transmission. Graft transmission is more rare and has been well documented in two cases of HSCT [7, 20]. In 2001, Rigau-Pérez et al. described a case of dengue in a HSCT recipient during the 1994–1995 dengue epidemics in

Puerto Rico. The patient, a 6-year-old child, died 11 days after transplantation and DEN4 was detected in blood, ascitic fluid and tissue samples. The donor developed fever 2 days after marrow harvesting and dengue was diagnosed by the presence of specific IgM antibodies. DEN4 serotype was confirmed in donor samples [7]. The other case of graft transmission was recently reported in a HSCT recipient from Germany whose unrelated donor had returned from Sri Lanka 3 days before donation. Despite the predictable risk, the recipient was in urgent need of the transplant and the transplant was consented through a Declaration of Urgent Medical Need [20]. Graft transmission was also reported but less well documented in a renal transplant recipient from Singapore whose living donor had acquired dengue 6 months before donation. As Singapore is an endemic area and dengue virus does not persist in the host for so long time after infection resolution, dengue was more likely to have been transmitted by mosquito bite [11].

Blood transmission: Dengue transmission by blood has been increasingly demonstrated as a consequence of the expanding areas of dengue transmission and more frequent outbreaks. The majority of dengue cases are well known to be asymptomatic. These individuals may offer to donate blood, and consequently viremic donors have been observed during outbreaks in all investigated areas [21–23]. The transmission of dengue to naïve blood recipients have also been detected during outbreaks. Recently, one case of blood-transmitted dengue was documented in a patient with severe aplastic anemia after platelet transfusion from a regular platelet donor who was asymptomatic at the time of donation, but seroconverted (both IgG and IgM) in the following month. The other recipient of the same platelet donation did not develop dengue [24].

9.2.1.3 Pretransplant Management

Dengue control is based on vector control and community-based programs to keep the environment free of potential breeding sources, mainly artificial water containers such as discarded tires, uncovered water storage barrels, flower vases, etc. The mosquitoes breed in limpid water. In endemic regions, transplant patients should receive information about dengue transmission and *Aedes* habits to avoid exposure. The incidence, morbidity and mortality of dengue fever and its complications are difficult to estimate, as no prospective seroepidemiological study has been performed in transplant recipients from endemic areas. Seropositive patients have a theoretical risk of developing severe dengue in case of reinfection during outbreaks. Pretransplant screening for dengue is currently not recommended, but prospective studies are necessary to evaluate the cost effectiveness of serological assessment before transplantation.

9.2.1.4 Posttransplant Management

Clinical findings: In general, case reports are more prone to describe severe cases of dengue in detriment of mild ones. Thus, among nine publications of dengue cases, six reported severe clinical presentations, DHF, or DSS [7–9, 11, 13, 18, 20]. On the other hand, in published case-series of dengue from Brazil (27 cases) and Singapore (6 cases), both in renal transplant recipients, a benign course was generally observed and death probably due to DSS was registered in 1 of the 27 patients (3.7%) from the Brazilian series [10, 12]. More recent case-series publications confirm that mild cases are more frequent than severe cases in solid organ and stem cell transplant recipients. In 2013, 102 cases of dengue in renal transplant recipients from Pakistan were retrospectively reviewed. Forty-four patients (43%) had primary and 58 (56.8%) had secondary dengue infection. Thrombocytopenia was seen in 95% of the cases, with a mean duration of 11 ± 9 days. Most of the patients presented fever (80%), which was less frequently seen in patients receiving high-dose steroids (>7.5 mg). DF occurred in 90 (88%), and DHF/DSS occurred in 12 (11.7%). High dose of steroid use had a negative impact in those with primary but not in secondary infection. Graft dysfunction occurred in 68 (66.7%) of the patients. Interestingly, patients on a CSA-containing regimen had significantly less severe disease [17]. Studies have shown that CSA can be a potential drug for the treatment of flavivirus infections [25].

Dengue mortality rates vary from 0.026% in classic dengue fever up to 5% in DHF in the immunocompetent population. In addition, the paucity of reports of DHF or DSS in transplant recipients may reflect the T-cell immunosuppression induced in this population and the consequent low inflammatory response.

Diagnosis: Before day 5 of illness, virus isolation, nucleic acid or antigen detection can be used to diagnose dengue. NS1 antigen detection kits can be used in laboratories with limited equipment and yield results within a few hours. At the end of the acute phase of infection, serology is the method of choice for diagnosis. IgM antibodies are the first immunoglobulin isotype to appear and are detectable in 50% of patients by days 3–5 after onset of illness, increasing to 80% by day 5 and 99% by day 10. IgM levels peak about 2 weeks after the onset of symptoms and then decline generally to undetectable levels over 2–3 months. Anti-dengue serum IgG is generally detectable at low titers at the end of the first week of illness, increasing slowly thereafter, with serum IgG still detectable after several months, and probably even for life [3].

Treatment: There is no specific antiviral drug and management of dengue should follow a stepwise approach including overall assessment (history, symptoms, physical examina-

tion, mental assessment, and laboratory tests), diagnosis, and assessment of disease severity. On the basis of evaluations of the history, physical examination and/or full blood count and hematocrit, clinicians are able to determine whether the disease is dengue, which phase it is in (febrile, critical or recovery), whether there are warning signs, the hydration and hemodynamic status of the patient, and if the patient requires admission. Hydration is the mainstay of dengue treatment. According to some case series, immunosuppression did not seem to affect the outcome and there is no evidence to recommend decreasing immunosuppression.

In conclusion, there should be a high index of suspicion for dengue illness in transplant recipients presenting with thrombocytopenia in endemic areas, even if afebrile. Prolonged thrombocytopenia (more than 10 days) is expected and has been observed in transplant recipients with dengue in comparison to general population (around 3 days). During epidemics, the cost benefit of screening organ donors and blood products in hyperendemic regions needs to be assessed, since studies conducted during epidemics have detected DEN viremia by PCR in 0.04–0.3% of in asymptomatic blood donors [21, 22].

9.2.2 Yellow Fever

Yellow fever (YF) is a vector-borne disease caused by yellow fever virus, a RNA virus belonging to the family *Flaviviridae*, genus *Flavivirus*, transmitted to humans by the bite of infected mosquitoes. YF virus infects humans and monkeys in three types of transmission cycle: sylvatic (jungle), savannah, and urban.

In the jungle cycle of South America, monkeys infected by sylvatic mosquitoes *Haemagogus* pass the virus to other mosquitoes that fed on them. Humans are sporadically exposed when enter the transmission cycle of the agent. In the savannah cycle, the virus is transmitted by semidomestic mosquitoes *Aedes* (mainly *Ae. africanus*) that infect both monkeys and humans and produce small-scale epidemics in African rural areas. In the urban cycle, monkeys are not involved and infection is transmitted among humans by the mosquito *Aedes aegypti*, generally causing explosive epidemics in populous areas [26, 27].

After an incubation period of 3–6 days, immunocompetent patients develop fever, muscle pain, headache, shivers, loss of appetite, nausea and vomiting, characterizing the acute phase of yellow fever. Most patients improve and symptoms disappear in few days. Around 90% of the cases are mild or asymptomatic. However, between 15 and 20% go into a severe toxic phase with reappearance of fever and the development of jaundice and sometimes bleeding. About 50% of patients in toxic phase die within 10–14 days [27].

9.2.2.1 Geographic Distribution

Over 900 million people are at the risk of being infected in the tropical areas of Africa and South America. Thirty three countries in Africa that are located between 15 latitude north and 10 latitude south of the equator, are at risk of yellow fever. Sudan is the only country in Eastern Mediterranean Region in the yellow fever zone. Large epidemics have been reported in Sudan in 1940, 1959, 2003, 2005, 2012, and 2013 [28]. In the Americas, from 1985 to 2012, 95% of all yellow fever cases were reported by four countries: Peru (54% of all cases), Bolivia (18%), Brazil (16%), and Colombia (7%). The other countries with conditions for yellow fever transmission are Argentina, Ecuador, French Guiana, Guyana, Panama, Paraguay, Suriname, Trinidad and Tobago and Venezuela. From 2000 to 2013, more than 1100 laboratory-confirmed cases were reported in the Americas, with the largest numbers reported from Brazil and Peru [29].

The annual estimated cases per year in at risk areas are 200,000, causing 30,000 deaths. Fatal cases of yellow fever have been reported among unvaccinated tourists from the United States and Europe who visited endemic areas of yellow fever [30, 31].

9.2.2.2 Transmission in Transplant Patients

No case of yellow fever has been reported in solid organ or stem cell transplant recipients. However, the occurrence of the disease in the transplant population in developing countries can be just a matter of time, in the case of urban yellow fever resurgence. The control of yellow fever is based on mosquito eradication and vaccination of people living in or traveling to risk areas.

9.2.2.3 Pretransplant Management

Since live-attenuated vaccines are contraindicated after transplant, SOT candidates from endemic regions are recommended to receive yellow fever vaccine (YFV) before transplant. This practice should be encouraged as pretransplant immunization elicits prolonged YF immunity in SOT patients, as recently demonstrated. A prospective study conducted in 53 SOT recipients (29 kidney, 18 liver, 1 heart, 2 kidney–liver, 2 pancreas–kidney, and 1 heart–liver) showed that protective YF antibody level was detectable in 98% of the recipients vaccinated before transplantation. The median time from vaccination to assessment of YF immunity was 13 years, varying from 3 to 32 years [32].

9.2.2.4 Posttransplant Management

The live-attenuated YFV is contraindicated in transplant recipients due to the potential risk of severe adverse events,

mainly encephalitis [30, 33]. Extremely rare cases of viscerotropic disease following YFV have been reported in general population [34].

Although contraindicated, some transplant patients may inadvertently receive the vaccine. Recently, an increase in the number of reported human YF cases occurred in Brazil, from 2 cases in 2006 to 46 in 2008. This increase prompted health authorities to expand the area of vaccine recommendation. These facts were widely reported by the media and caused a rush to health services to receive the vaccine, including some individuals for whom YFV is contraindicated. The safety of YFV in SOT recipients inadvertently vaccinated against YF was evaluated through a questionnaire sent to physicians affiliated to the Brazilian Society of Organ Transplantation. Nineteen SOT (14 kidneys, 3 hearts, and 2 liver) received YFV by mistake and only 1 patient had a slight reaction at the site of YFV injection. No one had systemic reactions or required hospitalization [35]. However, no recommendation for YFV safety can be made based on this data due to the small number of patients studied. SOT recipients should, if possible, not travel to endemic areas, and, if exposure is likely, the benefits of YF immunization should be evaluated and a decision should be taken carefully on a case-to-case basis [36].

A slightly different scenario is observed in HSCT recipients as immunosuppression is progressively decreased over time and live-attenuated vaccines may be used after 2 years of HSCT, if the patient is not receiving immunosuppressive drugs. Thus, YFV may be considered for HSCT recipients under those circumstances and living in areas of great risk of YF. In other situations vaccination is contraindicated and should be avoided [37].

More than 20 cases of YF vaccination have been reported in HSCT recipients without side effects [16, 38, 39]. In 2008, when an expanded area for YFV recommendation was determined in Brazil, a seroepidemiological survey was conducted in 27 HSCT recipients living in the expanded areas to identify those susceptible to YF. Information about previous vaccination was available for 24 of them. These patients underwent HSCT at a median of 406 days, ranging from 21 to 2334 days. Sixteen had received YFV before HSCT and persistent YF immunity was detected in 14 of them (87.5%). Seven patients (29.1%) were susceptible to YF despite the pretransplant vaccination in two of them. Three were within the first 2 years of HSCT, and consequently were not eligible to YF vaccination. The remaining four HSCT recipients received YFV without adverse events [16]. Interestingly, among the eight HSCT recipients who did not receive YFV before transplantation, three (37.5%) were immune. The presence of YF antibodies after HSCT may reflect passive immunity from the donor or recipient exposure to YF virus. Asymptomatic or mild cases of YF are common in immunocompetent hosts and may possibly also occur in HSCT recipients [16].

It is important to stress that vaccine strain viremia frequently occurs in healthy persons during the first week of primary YF vaccination. In booster doses, vaccine strain viremia is unlikely [30, 40]. In the case of donor vaccination, it is prudent to wait at least 1 week to stem cell or marrow harvesting or organ donation.

Transplant recipients from developed countries traveling to endemic regions of YF should be advised of the risks posed by such travel, instructed in methods for avoiding vector mosquitoes, and supplied with vaccination waiver letters by their physicians [30].

9.2.3 Measles

Measles virus belongs to the *Paramyxoviridae* family, which infects primate species, but can only be maintained in human populations. Measles is an acute illness and one of the most contagious diseases as almost all susceptible persons acquire the disease once exposed to the virus. The virus spreads from person to person within aerosol droplets generated during coughing. Measles tends to result in epidemics that cause many deaths, especially among young malnourished children. Characteristic symptoms such as fever, dry cough, coryza, rash, conjunctivitis and photophobia initiated 10 days after infection [41].

More than 20 million people are affected by measles each year. Poverty impacts lethality rates. While global measles deaths have decreased by 75% worldwide in recent years, from 544,000 deaths in 2000 to 146,000 in 2013, more than 95% of measles deaths occur in countries with low per capita incomes and weak health infrastructures [42].

Vaccination is the best way to control measles and has a major impact on measles deaths. A safe and effective live-attenuated virus vaccine is available for the past 40 years.

9.2.3.1 Geographic Distribution

Measles has not been eliminated from most countries and is the first disease to reappear when vaccine coverage falls. As a result of decreased measles vaccination coverage, developed countries have also registered epidemics of measles in recent years: Ireland and the Netherlands in 2000, Korea in 2001, Italy in 2002, Switzerland in 2006, Japan in 2007, Austria and United States in 2008. During 2011, 115 measles outbreaks were reported in 36 of the 53 member states in the European Region. More than 26,000 measles cases had been reported as of October 26th 2011. France reported the largest number of cases (>14,000); these occurred predominantly among older children and young adults who had not been vaccinated or whose vaccination history was unknown. The primary reason for the increased transmission and outbreaks of measles in Europe is failure to vaccinate [43]. Although measles elimination has been declared in the US in 2000, a

total of 159 cases were reported from January 4th to April 2nd, 2015 [44]. In 2014, more than 96,000 cases of laboratory-confirmed measles were reported in all WHO regions.

9.2.3.2 Transmission in Transplant Patients

Measles has been diagnosed in transplant patients as a result of community outbreaks or recent travel to endemic or epidemic regions. As measles epidemics resurge, susceptible transplant recipients from developed and developing countries become an easy target for measles and its complications. Previous and/or donor-transferred immunity wanes over time and measles antibodies titers are expected to fall below the protective level during follow-up. Up to 55% of HSCT and 22% of SOT recipients lose measles immunity by 24 and 6 months after transplant, respectively [45, 46].

Protection against measles in transplant recipients strongly relies on herd immunity, since live-attenuated vaccines have restricted use in this population. In SOT recipients, measles vaccination is recommended before the transplant, and in the setting of HSCT, measles vaccine can be safely used after 24 months of transplantation in patients not receiving immunosuppressive drugs [47].

The largest series of measles cases in transplant population was described in HSCT recipients during an outbreak in Brazil. In view of the limitations to the use of live-attenuated vaccines in these patients, a serological survey was performed and identified 54 susceptible to measles, 8 of which acquired measles in the first 3 months of the outbreak (attack rate 14.8%). One patient developed measles pneumonia. No patient developed CNS complications during follow-up or died of measles [45]. To interrupt measles transmission among susceptible patients, a protocol of early measles vaccination (1 year after HSCT) was successfully instituted and no more cases were seen up to the end of the outbreak which lasted 2 years [48]. During the 2000–2001 measles epidemics in Korea, one fatal case of measles pneumonia was reported in a 39-year-old HSCT recipient with chronic GVHD [49].

In the setting of SOT, five cases of measles-associated encephalitis have been reported in renal transplant recipients, at a median of 5 years after transplantation (range 3–11 years) [50–53]. Preceding measles infection was reported in three of the five patients, 6 weeks to 4 months before the appearance of neurological symptoms. In the remaining two patients, measles diagnosis was retrospectively performed by serology in one case and by necropsy in the other case. Three patients had received one dose of measles vaccine in infancy, one had not been vaccinated and in the remaining one, vaccination status was not informed. Three patients (60%) died as a consequence of CNS complications and the other two patients survived with sequelae [53]. More recently, a case of measles was diagnosed in a 31-year-old liver transplant recipient during an outbreak in public schools in Salzburg, Austria. Liver biopsy revealed portal inflammation and endothelialitis as well as alteration of the cholangium suggestive of mild acute transplant rejection triggered by measles infection [54].

9.2.3.3 Pretransplant Management

Measles is difficult to eradicate as sustained elimination requires the maintenance of more than 90% one-dose coverage among preschool children and more than 95% two-dose coverage among school-aged children [55]. Due to decreased coverage rates of measles vaccination, the disease has reappeared in developed countries.

Facing this scenario, vaccination status for measles should be checked and updated in patients awaiting SOT. The recent Infectious Diseases Society of America (IDSA) guideline states that live-attenuated vaccines can be administered to SOT candidates at 6–11 months of age if they are not immunosuppressed and if the transplantation is not anticipated in the ensuing 4 weeks [56]. However, the presence of maternal antibodies may interfere in the antibody response. A recent study in liver transplant candidates vaccinated prior to transplantation, showed a 12.5% rate of sustained immunity after transplant in children who received measles vaccine before 12 months of age and 63.6% in those older than 12 months [57].

9.2.3.4 Posttransplant Management

In addition to the pretransplant assessment of immunity and vaccination update, clinicians should have in mind that antibody levels decline rapidly in the first 12 months following transplantation. Despite the restricted use of measles vaccine in transplant populations, there is growing evidence of its safety in situations where the risk of measles complications outweighs the risk of adverse events of vaccination. Taking into account the current epidemiological situation of measles worldwide, regular monitoring of posttransplant antibody levels to measles should be considered in transplant recipients, and if loss of protective antibody level is detected, reimmunization might be an option. Several groups have reported that post-transplant measles vaccination could be efficacious and safe [58–60].

Measles vaccine administered after the 12th month of transplant was safe and effective during the 1997–1999 measles epidemics in Brazil. Around 53% of the patients were receiving immunosuppressive drugs. No moderate or severe adverse events were observed and vaccination was the strategy used to interrupt measles transmission among susceptible HSCT recipients [48].

The safety of measles vaccine beyond its recommended use has also been evaluated after solid organ transplantation. In two studies recently reviewed [61], 31 patients received

measles vaccination between 1.5 and 65 months after transplantation. No adverse event was noted among 29 children. In the remaining two children (6.4%), signs of organ rejection were observed 3 weeks after first vaccination, but the possible association of vaccination and rejection remains elusive. Seroconversion rates were higher in patients who had received measles vaccine before transplant (85%) as compared to children immunized only after transplantation (41%). Similarly, sustained immunity was observed in 64% of the patients previously vaccinated in comparison to 29% of the patients who were vaccinated only after transplantation [61–63]. In another study, overall responses rates of 73% were observed in 26 children receiving primary measles vaccination after liver transplantation. However, 18 patients required multiple doses [58].

Thus, although transplant guidelines do not recommend live-attenuated vaccines after solid organ transplantation, some groups have indicated measles, rubella, mumps and varicella vaccines in patients fulfilling the following criteria: (1) at least 1 year of transplant and stable general condition; (2) no use of systemic steroids to treat acute rejection within the last 6 months; (3) exclusive use of tacrolimus as an immunosuppressive drug, and serum through concentration of tacrolimus <5 ng/mL (indicating that rejection reaction was under control); (4) no severe immunosuppression according to blood examinations; and (5) provision of written informed consent from all patients or guardians [64].

In conclusion, compliance to vaccination guidelines is strongly recommended in both SOT and HSCT recipients. Periodical evaluation of measles immunity identifies susceptible patients eligible to revaccination. During epidemics, clinicians should have a high index of suspicion for measles in transplant recipients with respiratory symptoms, as rash may be absent or atypical. In epidemic situation when the risk of measles complications may overweigh the risk of adverse events, measles vaccination may be considered in individuals receiving immunosuppressive drugs.

9.2.4 Rabies

Rabies is a zoonotic viral disease which infects domestic and wild animals. Rabies virus causes acute and progressive encephalitis. It is transmitted to humans through close contacts with saliva from infected animals, usually a dog, cat, raccoon, skunk, mongoose, or bat. Domestic dogs cause over 99% of all human deaths from rabies. Bites are the most common mode of contamination, even though the contact of infected saliva with scratches, licks on broken skin and mucous membranes may also cause rabies [65, 66]. After the virus is inoculated into humans, it is taken up through peripheral nerves and infects the central nervous system causing encephalitis. This process may take weeks or months. The first symptoms of rabies are usually nonspecific and suggest involvement of the respiratory, gastrointestinal and/or central nervous systems.

An average of 60,000 people die from rabies annually, and more than 15 million people receive postexposure prophylaxis every year. Bat rabies variants of genotype 1 have different tissue tropisms and patients with bat lyssavirus variants may show clinical manifestations different from what is seen in humans infected with canine rabies.

9.2.4.1 Geographic Distribution

Rabies is widely distributed on all continents except Antarctica. Most industrialized countries have eliminated rabies from domestic dog populations. However, in the majority of developing countries, rabies remains endemic, poorly controlled and certainly underreported [67]. The actual incidence is unknown as several countries do not include rabies among notifiable diseases. Dog bites are the most frequent mode of transmission in most countries of Africa, Asia, Latin America, and the Middle East. In contrast, in North America, most documented human rabies deaths occurred as a result of infection from bat rabies virus [68].

9.2.4.2 Rabies in Transplant Recipients

The transmission of rabies virus through cornea transplantation has been described, but transmission through solid organ transplantation was not recognized before 2004. Seventeen cases of rabies have been reported in transplant recipients, and to date, all have been transmitted through the transplanted tissue or organ. Nine cases followed corneal transplantation, including eight deaths.

Two clusters (Texas in 2004 and Germany in 2005), totaling seven rabies cases, have occurred following SOT [69, 70]. Each of the donors died of an illness compatible with or proven to be rabies, even though the diagnosis was only suspected when the recipients died of rabies [71]. These cases followed the transplantation of liver, lung, kidney, kidney-pancreas, and iliac artery grafts. Donor-transmitted rabies may have a long incubation period. Patients developed encephalitis between 30 and 60 days post-transplant, and all symptomatic patients died. Patients in Germany received postexposure prophylaxis (PEP) and antiviral treatment, though not until postoperative day 45. The liver recipient in this cluster had been previously vaccinated (around 20 years before) and never developed disease [69].

In the SOT cases, the donors had a recent history of bat and dog bite which were not elicited or considered important. Since bat teeth are very fine, bat bites may be undetectable and the epidemiological risk underestimated. Recently, the investigation of a donor-transmitted rabies to a kidney transplant recipient highlighted the donor contact

with raccoons and wildlife [72]. Thus, a detailed history of bites, in contact with bats and wildlife should be obtained from organ donors' relatives and friends. In the absence of a clear history, physical signs and reliably performed rabies diagnostic tests, the safest strategy is to exclude any donor with neurological symptoms and signs.

9.3 Bacterial Infections

9.3.1 Tuberculosis

Tuberculosis (TB) remains a major global health problem, ranking as the second leading cause of death from an infectious disease worldwide after the human immunodeficiency virus (HIV).

Although the TB incidence rates (new cases per 100,000 population per year) are decreasing in most parts of the world and the TB mortality rate (deaths per 100,000 population per year) has fallen by 45% since 1990, the numbers are still impressive. The latest estimates show that there were 9.0 million new TB cases in 2013 (13% of those among people living with HIV) and 1.5 million TB deaths (1.1 million among HIV-negative people). Most of the estimated number of cases in 2013 occurred in Asia (56%) and the African Region (29%); smaller proportions of cases occurred in the Eastern Mediterranean Region (8%), the European Region (4%) and the Region of the Americas (3%). India and China alone accounted for 24% and 11% of global cases, respectively [73].

Since the rate of TB cure is lower among HIV-positive patients (74%) in comparison to HIV-negative (88%), transmission is facilitated in places where HIV infection is highly prevalent [73]. Other immunocompromised hosts, including solid organ and stem cell transplant recipients, are also more prone to reactivation of *Mycobacterium tuberculosis* infection [74].

One of the components of the WHO program for TB elimination is the recognition and treatment of latent tuberculosis infection (LTBI). There is clear evidence of benefit from systematic testing and treating of LTBI in the following groups: people living with HIV, adult and child contacts of pulmonary TB cases, patients preparing for organ or hematologic transplantation, patients initiating anti-TNF treatment, patients receiving dialysis and patients with silicosis [75]. Among transplant patients, lung transplant recipients have the highest risk, although the local incidence of infection better defines the risk rather than the organ transplanted [76].

The six countries that stand out as having the largest number of TB incident cases in 2013 were India (2.0–2.3 million), China (0.9–1.1 million), Nigeria (340,000–880,000), Pakistan (370,000–650,000), Indonesia (410,000–520,000) and South Africa (410,000–520,000) [73]. Almost all developing countries with active transplant programs, such as Argentina, Brazil, China, Egypt, India, Iran, Nigeria, Pakistan, South Africa, Taiwan, Thailand, Turkey, among others, have reported TB cases in transplant recipients, nicely reviewed in some publications [77–79]. The majority of the reports are in solid organ transplant recipients, especially after renal transplantation.

9.3.1.1 Transmission in Transplant Recipients

Tuberculosis among transplant recipients may arise from reactivation of quiescent foci of *M. tuberculosis*, transmission by the graft or contamination by actively infected persons. Graft transmission has been documented in renal, lung and hepatic transplantation, but accounts for less than 5% of all TB cases in transplant recipients [76, 77]. Cross-transmission of TB has led to an outbreak in a renal transplant unit but also appears to be a rare event [80]. Reactivation of LTBI accounts for the vast majority of TB cases reported in transplant recipients, and largely reflects the local incidence. In North America, the prevalence of LTBI among renal transplant recipients is 0.5–1%, in Northern Europe is 1–4%, and can reach 15% in India and Pakistan [81, 82].

Few studies have adequately described the incidence rate in the transplantation setting. The risk of TB in transplant recipients is estimated to be 20–50 times higher than in general population, even in developed countries. In Europe, recent series have shown an incidence varying from 0.45 to 0.9% [83, 84]. The highest incidence (2.6–6.4%) is observed in lung transplant recipients [85, 86]. Although mortality rate may have decreased due to better diagnostic techniques, it remains high varying from 9.5 to 17% in some series [79].

9.3.1.2 Pretransplant Management

Epidemiological risk: In recent years, a growing number of transplant programs have been implemented in developing countries where prevalence rates of TB are high. In these places, prompt investigation of epidemiological risk for LTBI is mandatory. Previous TB infection or contact with infected persons should be particularly investigated as well as careful radiological evaluation to check for images suggestive of previous healed TB.

Diagnosis of LTBI: LTBI can also be evaluated through the tuberculin skin test (TST), a delayed type hypersensitivity reaction to intracutaneous injection of antigens isolated from culture filtrate by protein precipitation. The sensitivity of the TST is lower in immunocompromised hosts and induration ≥5 mm is considered positive [75]. The specificity of TST in transplant patients from developing countries is also impaired because the delayed type hypersensitivity reaction may indicate infection with non-tuberculous mycobacteria or previous vaccination with the Bacille Calmette-Guérin (BCG), a live-attenuated mycobacterial strain derived from *Mycobacterium bovis* [87]. The role of TST as well as the new interferon gamma release assays (IGRAs) in the evaluation of LTBI in transplant recipients has not been fully investigated and prospective studies evaluating the predic-

tive value of those tests in the development of tuberculosis are lacking. A recent prospective study comparing TST and IGRA in HSCT candidates showed a substantial agreement between tests (Kappa index = 68.5%) [88].

The indication for screening with TST and/or IGRA should be guided by local incidence of TB in transplant recipients. Preventive chemotherapy against TB without prior screening for LTBI may be appropriate for all transplant candidates in regions of high TB incidence (≥100 per 100,000 population). In regions of medium TB incidence (≥20 per 100,000 population or in regions with medium local TB burden), all candidates should be screened for the presence of *M. tuberculosis* specific immune responses. In regions of low TB incidence, a decision for screening of transplant recipients for the presence of *M. tuberculosis* specific immune responses should include an individual risk assessment for LTBI [89].

Donor screening: While the majority of posttransplant TB occurs secondary to reactivation in recipients with unrecognized or untreated LTBI, transmission of *M. tuberculosis* through the allograft can occur. The management of TB in all organ donors requires accurate historical, clinical and laboratory data, which can be challenging especially with deceased donors. The following general recommendations were proposed by the Consensus Conference Report of donor-derived infections, endorsed by American Society of Transplantation, Canadian Society of Transplantation and The Transplantation Society [76]:

- Rule out active TB in the donor with any identified historical or epidemiologic risk factors. For suspected or confirmed TB, donation should be deferred except in dire circumstances.
- All SOT donors should have a careful epidemiologic and personal medical history, physical and a chest radiograph.
- TST and IGRA should be cautiously interpreted taking into account the epidemiologic history and chest radiograph findings. A negative result on TST or IGRA does not rule out active TB.
- For lung donors, bronchoscopy specimens should be obtained for mycobacterial testing for TB and atypical mycobacteria (AFB smear and culture) prior to donation.
- Molecular methods for mycobacterial culture and species identification are preferred to standard culture if available, due to the shorter turn-around time.
- There is insufficient evidence to recommend IGRA testing of all SOT donors. Further research is needed, especially in deceased donors where the tests have potential utility for identification of moderate or high risk of TB donors.
- Donation need not be deferred for the diagnosis of LTBI in any SOT donor including lung donors.
- Urinalysis with microscopy, genitourinary imaging and urine AFB smear and culture should be considered for organ donors in intermediate and high-risk countries, especially for kidney donors.

Prophylaxis: INH prophylaxis reduces the risk of development of active disease [90, 91]. All candidates from regions of high TB incidence (≥100 per 100,000 population) or those screened with a positive TST or IGRA test should receive preventive chemotherapy to reduce the risk for the development of TB. A preventive chemotherapy regimen of isoniazid 5–10 mg/kg *per* day for 6–9 months or longer is recommended [89, 91]. Alternative schedules may be considered on individual basis.

Fear about toxicity has hampered the implementation of INH prophylaxis especially in the setting of liver transplantation. However, previous and recent studies have shown that INH prophylaxis is effective and safe also in this population [92–95]. At most, toxicity is similar to expected in general population (less than 20%) with normalization of liver function tests after drug discontinuation [96]. A recent meta-analysis demonstrated that isoniazid prophylaxis was associated with reduced reactivation of tuberculosis in liver transplant recipients at risk for TB (0.0% versus 8.2%, $p = 0.02$), and isoniazid-related hepatotoxicity occurred in 6% of treated patients, with no reported deaths [90]. In general, liver biopsies often show chronic rejection, recurrent hepatitis C, or other causes when INH toxicity is suspected [97].

Living donors with a positive TST or IGRA should also be offered treatment for latent TB prior to donation. As completion of this treatment may delay the transplant and adversely impact the recipient, the prophylaxis need not be completed before the transplant occurs. In this case, the recipient should also receive INH prophylaxis [76].

9.3.1.3 Posttransplant Management

Risk factors and clinical findings: In areas of high prevalence of TB, a high index of suspicious should be kept in the first months after transplantation. About one-third to one-half of all cases of active TB after solid organ transplantation are disseminated or occur at extrapulmonary sites, compared to about 15% of cases in immunocompetent persons [76]. In HSCT recipients, lung is the organ more frequently affected [16, 95, 98, 99]. Extrapulmonary forms include renal, ganglionar, pleural, central nervous system, bone marrow, and miliary TB [100].

Risk factors for TB in SOT recipients include pulmonary images suggesting previous TB infection, the use of T-cell depleting antibodies, enhanced immunosuppression in the setting of rejection, chronic renal insufficiency or hemodialysis for kidney transplant recipients, diabetes mellitus, hepatitis C virus infection for kidney transplant recipi-

ents, chronic liver disease, or increased recipient age [77, 78, 84, 89, 101, 102]. By multivariate analysis, the RESITRA (Spanish network of infection in transplantation) identified recipient age (RR, 1.05; 95% CI, 1.0–1.1) and receipt of a lung transplant (RR, 5.6; 95%, 1.9–16.9) as independent risk factors for tuberculosis. In the setting of HSCT recipients, the intensity of immunosuppression, unrelated or mismatched related transplants, acute or chronic graft versus host disease (GVHD) and total body irradiation increase the risk of TB reactivation after HSCT [89, 95, 98].

Diagnosis and treatment: Diagnosis of TB is based on clinical grounds and laboratory confirmation by bacilloscopy and culture, and real-time PCR. Novel generations of automated nucleic acid amplification tests, such as the Xpert MTB/RIF test (Cepheid, CA, USA), could potentially improve the rapid diagnosis of TB in transplant patients. WHO currently recommend this test as the initial diagnostic test for adults and children suspected of having HIV-associated TB, in substitution to conventional microscopy and culture [73]. Treatment of TB is made for at least 6 months, with three (isoniazid, rifampicin, and pyrazinamide) or four drugs depending on the rate of isoniazid (INH) resistance. Ethambutol should be added if the rate of INH resistance is greater than 4%. A longer duration of therapy (12 months) has been recommended for the treatment of miliary disease, bone and joint disease and meningitis in infants and children. Also, longer treatment should always be considered if the response to treatment is slow and is mandatory if second-line drugs are used to replace first-line drugs.

The field of tuberculosis in transplant populations is rapidly evolving and some of the current worries will probably be answered in the next years. However, it is important to highlight the unsatisfactory compliance with the LTBI preventive recommendations, as shown by literature data. A recent study demonstrated that about one-third of the transplant recipients did not complete the screening for LTBI and isoniazid prophylaxis was given to only 46% of the high-risk patients [91]. Previous studies have already expressed the same concerns [84, 98].

9.3.2 Leprosy

Leprosy is a chronic infectious disease that affects the skin, the peripheral nerves, eyes, and upper respiratory tract mucosa. The infection is caused by *Mycobacterium leprae*, an acid-fast rod-shaped bacillus discovered by G.A. Hansen in 1873. Humans are the principal reservoir of *M. leprae* and the disease spreads by aerosolized droplets from multibacillary patients, and less commonly through direct skin contact [103]. Recent studies have suggested that direct contact with wild armadillos may contribute to the transmission of leprosy in some areas [104, 105].

The onset of leprosy can be insidious and the disease may be difficult to recognize. In a susceptible host, a skin lesion may develop after an incubation period averaging 2–4 years, varying from 3 months to 40 years [103]. This initial phase is called intermediate leprosy and in many patients the lesions can heal spontaneously or progress to a clinical spectrum depending on the host immune response to *M. leprae*.

According to the type of T-cell response, the granulomatous spectrum of leprosy is classified in tuberculoid (TT), characterized by few skin lesions and low bacterial loads, borderline tuberculoid (BT), borderline (BB), borderline lepromatous (BL) and lepromatous (LL), characterized by diffuse skin lesions and high bacterial loads [103]. A predominant T-helper 1 (Th1) response to *M. leprae* is encountered in lesions of patients with TT and BT forms, in contrast to the Th2 profile found in disseminated lesions of patients with BB, BL, and LL forms of leprosy. Patients are classified as having one of two leprosy types, paucibacillary (PB) or multibacillary (MB). Classifying leprosy is important to ensure appropriate treatment particularly for the MB form of the disease [106].

9.3.2.1 Geographic Distribution

The vast majority of the world's population is not susceptible to leprosy. Susceptibility appears to be determined by genetic characteristics [107]. Since the introduction by the WHO Leprosy Elimination Program of multidrug therapy (MDT) free of charge to all patients worldwide, most previously highly endemic countries have reached leprosy elimination (registered prevalence rate < 1 case per 10,000 population) [108]. Since 2000, the focus has moved from leprosy prevalence to incidence of new cases. A dramatic and sudden decline in new case detection of over 60% was observed from 2001 to 2005. However, the new case detection trends between 2010 and 2013 are remarkably stable suggesting stagnation in leprosy control [109]. Among the 18 countries reporting more than 1000 cases in 2011, India, Brazil, and Indonesia have the highest number of cases. Trends in new-case detection from 2004 to 2011 show that India and Brazil had a very slow decline since 2006 and 2007, respectively. Indonesia, after a plateau since 2006, had a significant increase in 2011. These three countries represented 83% of the new cases detected in 2011, with India contributing 58%, Brazil 16%, and Indonesia 9% [110].

9.3.2.2 Transmission in Transplant Recipients

In SOT recipients, 25 cases of leprosy have been described, 21 in renal, 3 in heart and more recently, 1 case was reported in a liver transplant recipient [111–115]. All cases occurred in patients who were living or had lived in leprosy endemic areas. The majority of the cases was exposed to *M. leprae* many years before transplantation and developed leprosy 5

months to 12 years after transplantation. In the scenario of HSCT, six cases of leprosy were reported in Brazil, all in allogeneic HSCT recipients [116]. Three patients did not report a previous history or had contact with persons with leprosy, and the source of *M. leprae* was unknown. The remaining three had leprosy before HSCT and the disease reappeared after transplant, two cases as BL and one as TT leprosy.

9.3.2.3 Pretransplant Management

Epidemiological risk: Leprosy should be inquired in transplant candidates and donors who live or have lived in endemic areas. A pro-active attitude to get information on epidemiological risks is needed to overcome social stigmas related to leprosy [117].

9.3.2.4 Posttransplant Management

Clinical findings: So far, there are no guidelines for the management of leprosy in transplant recipients. Physicians need to be trained in the differential diagnosis of skin lesions in their patients and to identify at least two cardinal signs of leprosy: anesthetic skin lesions and enlarged nerves [112]. This involves training, supervising and monitoring primary healthcare staff as well as continued education. Diagnosis based only on an anesthetic patch is likely to miss about 30% of the MB cases [108]. Leprosy should be investigated in all patients with erythema nodosum, especially in endemic areas; although leprosy cases have also been reported from areas of low endemicity [118].

Treatment: PB leprosy should be treated with the combination of rifampicin and dapsone (PB multidrug therapy or PB-MDT) for 6 months, and MB leprosy should be treated with the triple drug combination of rifampicin, dapsone and clofazimine (MB multidrug therapy or MB-MDT) for 24 months. According to the WHO recommendation, the rifampicin and part of the clofazimine component should be taken monthly under supervision.

Apparently, immunosuppression did not adversely affect the treatment of leprosy in most SOT patients. While most prescribed MDT as recommended by WHO for multibacillary disease, alternative drug schedules have been used because of fear of adverse effects of clofazimine and rifampicin [111, 119]. Other drug schedules include minocycline, moxifloxacin, and ethionamide [111, 118]. However, minocycline and ofloxacin should only be recommended in presence of adverse effects or drug interactions.

In the scenario of HSCT, 2 of the 6 patients responded to the treatment, even though a tempestuous course was observed in one of them. The remaining patient died soon after HSCT due to MRSA pneumonia and the response to the treatment could not be evaluated. Two patients (LL and BL forms, respectively) had a good response to the treatment, and in the remaining one (BT), the skin lesions had not healed after 8 months of clofazimine. Similar to SOT leprosy cases, alternative drug schedules were used in the HSCT recipients to avoid possible adverse events [116].

Almost 30% of MB patients develop reactions, which are acute immunological phenomena that occur during the normal course of the disease. Reactions can be disastrous as they cause acute nerve damage. It is important to recognize reactions early and initiate treatment with steroids that improves outcomes for about 50% of patients. It is essential that the primary healthcare staffs are trained to recognize and treat reactions early [112].

9.4 Protozoan Infections

9.4.1 Chagas Disease (American trypanosomiasis)

Chagas disease is caused by *Trypanosoma cruzi*, a protozoan parasite firstly described by Carlos Chagas, in 1909. The parasite is generally transmitted by feces of infected triatomine insects through penetration of the parasite into the bite wound, conjunctiva or other mucous membrane [120]. Transmission can also occur from mother to child, by organ transplantation, blood transfusion, laboratory incident, and more recently, some outbreaks due to the ingestion of contaminated food or drink have been reported [121, 122].

9.4.1.1 Geographic Distribution

Chagas disease is endemic in the Americas, from the south of the United States to the south of Latin America. In areas with intra-domiciliary vector transmission, typically children <5 years of age are infected [123]. In the last two decades, the countries more affected by the zoonosis developed successful programs to reduce vector and blood transmission.

In 1991, the Southern Cone Initiative against Chagas disease proposed the interruption of transmission by the elimination of domestic vectors and the screening of blood donors in Argentina, Brazil, Chile, Paraguay and Uruguay. Uruguay was declared free of Chagas disease transmission by *Triatoma infestans* in 1997, Chile in 1999 and Brazil in 2006. Transmission has been effectively eliminated also in substantial areas of Argentina, Bolivia and Paraguay. Similar initiatives took place in 1997 for the Central America and Andean Pact countries, and in 2004 for the nine countries of the Amazon basin [124]. Thus, global disease prevalence has been reduced from the 1990 estimates of 16–18 million people infected six million to seven million worldwide, mostly in Latin America [124, 125].

9.4.1.2 Transmission in Transplant Recipients

Although all modes of transmission are observed in endemic and non-endemic areas, transplant recipients from non-endemic countries are more likely to acquire *T. cruzi* infection through blood transfusion or by an infected graft, whereas infected recipients from endemic regions are at risk of reactivation of latent infection during immunosuppression.

Currently, migration within and beyond endemic countries is the main factor in Chagas disease transmission. In 2006, after a series of reports of blood- and donor-transmitted Chagas disease, the US Food and Drug Administration approved the Ortho *T. cruzi* ELISA Test System to screen blood donors in the United States. Two positive tests are necessary for serological confirmation of *T. cruzi* infection. In case of test disagreement, a third technique is recommended. By mid-2008, more than 500 confirmed *T. cruzi*-infected donations were reported [126].

Thus, both in endemic and non-endemic areas a careful investigation of the epidemiological risk of latent Chagas disease is mandatory. Are at great risk of *T. cruzi* infection: (1) Native population from endemic areas; (2) population who have received blood transfusion in endemic areas; (3) offspring of a mother who are native from endemic country; (4) population who have lived in an endemic area for more than 3 months [127–129].

Blood-borne transmission: Blood transmission is considered as the second most important method of acquiring Chagas disease. With the exception of blood derivatives, all blood components are infective. Transmission by blood transfusion was first suggested by Mazza in Argentina in 1936, and the first cases of transfusion-associated Chagas disease were published in Brazil in 1952. The true number of reported cases is possibly underestimated since no more than 350 cases have been published [130]. In non-endemic countries, cases of transfusion-associated transmission can go undetected because acute infections are often asymptomatic and the level of awareness of Chagas disease among clinicians is low. In endemic countries where mandatory screening tests have been implemented since 1991, the residual risk of infection is calculated to be around 1:200,000 units [131].

Transmission via graft: The first strong evidence of transmission via a graft was reported in Brazil in 1981 [132]. Four renal transplant recipients developed Chagas disease; all four had received grafts from infected donors in chronic phase of *T. cruzi* infection [133]. Other series have been published, mostly from Argentina and Brazil, which perform the greatest number of renal and liver transplants in South America [134, 135]. In the US, from 2001 to 2011, transplant transmission of *T. cruzi* was confirmed in 9 of the 19 recipients of organs from 6 donors. The median interval from transplantation to diagnosis of infection was 8 weeks (range 3–29 weeks) [136]. Although uninfected recipients who receive an organ from a *T. cruzi*-infected donor may develop acute *T. cruzi* infection, transmission under these circumstances is not universal and appears to vary by organ type. According to the reported series, prospective monitoring and prompt treatment seems to be a safe and effective strategy and is currently recommended rather than prophylaxis [136, 137]. It is important to highlight the results of a recent randomized study comparing benznidazole versus posaconazole in the treatment of patients with chronic Chagas disease and positive *T. cruzi* PCR. A sustained parasitic response (for at least 120 days after the end of treatment) was observed in the benznidazole group. By the end of follow-up, 94% of the patients receiving benznidazole were PCR negative in comparison to only 20% of the patients receiving high dose of posaconazole ($p<0.001$) [138]. Thus, it seems reasonable to treat Chagas positive living donors with benznidazole for 30–60 days before donation to decrease the risk of transmission [128].

Reactivation: Reactivation may occur in *T. cruzi*-infected patients receiving immunosuppressive drugs. Risk of Chagas reactivation is higher after heart transplantation (up to 75%). A study from Brazil evaluated the risk factors for Chagas' disease reactivation, which occurred in 26.5% of previously infected heart transplant recipients. Multivariate analysis showed that the number of rejection episodes, neoplasms, and use of mycophenolate mofetil were the risk factors independently associated with reactivation [139]. In Argentina, where blood monitoring is routinely performed in SOT and HSCT recipients with previous *T. cruzi* infection, reactivation rates of 9–16% have been observed in kidney transplantation, 50–100% in heart transplantation, and 17% and 40% in autologous and allogeneic HSCT, respectively [140].

Pretransplant treatment in transplant candidates with Chagas disease has been suggested by some authors [120]. However, no study has evaluated if treated candidates have a decreased risk of reactivation after transplantation. Moreover, experts from endemic countries who have managed Chagas disease patients for decades consider that *T. cruzi* monitoring in blood, followed by preemptive introduction of benznidazole is a safe and effective strategy [140].

9.4.1.3 Pretransplant Management

Epidemiological risk: The investigation of the epidemiological risk of Chagas disease in donor and transplant candidates is mandatory. The following questions are suggested: Was the donor/recipient born in Latin America (South America, Central America or Mexico)? Was the donor/recipient born in, or did s/he spend significant time in Latin America? Was the donor's mother born in Latin America? [129].

Serological tests: In endemic areas, serological tests for *T. cruzi* are routinely performed. Due to the increase of migrant population in non-endemic areas, this routine has been extended to those locations as well. Two positive results with different serological techniques are necessary to consider a patient to be infected. If the tests are not available, posttransplant monitor-

ing is strongly recommended in transplant recipients with epidemiological risk of *T. cruzi* infection.

Management of T. cruzi-infected donors: Individuals who died of acute Chagas disease should be excluded from donation. Cadaveric donors chronically infected by *T. cruzi* are excluded from donation of heart and intestines. In the case of cardiac transplantation, the use of a heart from a patient with chronic Chagas disease is an absolute contraindication due to the risk of chagasic myocarditis during the period of immunosuppression. Other organs can be accepted provided that a close monitoring is ensured, preferably with molecular methods (PCR). Living donors chronically infected by *T. cruzi* should receive benznidazole 5–7.5 mg/kg orally for 30–60 days before donation. A careful monitoring of the donor is necessary due to benznidazole-related adverse events [128]. If *T. cruzi* infection is confirmed or suspected in the donor, all recipients should provide appropriate informed consent concerning the risk of receiving a potentially infected organ [128, 129].

9.4.1.4 Posttransplant Management

Monitoring of T. cruzi infection or reactivation: In case of positive Chagas serology in the donor or transplant candidate, parasitemia surveillance is required after transplantation to prompt detection of *T. cruzi* primary infection or reactivation. Hemoculture, microhematocrit, and the Strout method can be used, but currently quantitative PCR is the method of choice [132, 141]. Monitoring for acute infection is recommended weekly for 2 months, every 2 weeks for the third month, and then monthly until at least 6 months post transplantation [126]. Few modifications such as biweekly surveillance until the sixth month and yearly thereafter have been proposed by other groups [128]. If the serologic status of recipient or donor is not available, monitoring is indicated in native population from endemic areas (Latin America countries), in population who received blood transfusion in endemic countries or who have lived in an endemic area for more than 3 months [128].

Diagnosis and treatment of Chagas disease: In the diagnosis of posttransplant *T. cruzi* infection, the Strout method and PCR are more frequently used. Serological tests are only useful in seronegative patients who have received an organ from a seropositive donor (seroconversion). In recipients already infected by *T. cruzi* (positive pretransplant serology), a positive result in the Strout method is considered as a sign of reactivation. In the case of PCR, a positive result in patients with previous negative PCR is considered as a sign of reactivation. However, if the previous PCR was also positive, reactivation is determined by the increase in the parasitemia level [128]. In symptomatic patients, parasitological tests may be performed on samples from skin lesion biopsy or endomyocardial biopsy or on cerebrospinal fluid (CSF). Among transplanted patients, the most common symptoms of acute Chagas disease are subcutaneous nodules (chagoma), panniculitis, and myocarditis with signs of heart failure, fever, meningitis, encephalitis, and stroke. Prompt treatment with benznidazole or nifurtimox should be started if parasitemia is detected. The standard course for adults consists of benznidazole 5–7.5 mg/kg orally for 30–60 days or nifurtimox 8–10 mg/kg for 90–120 days.

The recommendation for prophylaxis is controversial both in cardiac or other transplant recipients [140, 142]. Protocols that recommend prophylaxis with benzonidazole both pre- and immediately posttransplantation, do not have clear advantages according to the clinical experience [143]. In other transplant recipients, preemptive treatment should be started if there is evidence of reactivation.

9.4.2 Leishmaniasis

The term leishmaniasis designates a group of diseases caused by the genus *Leishmania*. Leishmaniasis is primarily a zoonotic infection, which includes animal reservoir hosts in the transmission cycle. Anthroponotic forms of leishmaniasis have been increasingly observed as humans enter the transmission cycle of the parasite and get infected. In anthroponotic forms, man is the sole source of infection for the vector.

The protozoan is transmitted to humans through the bite of an infected female mosquito from the genus *Phlebotomus* (in the Old World) or *Lutzomya* (in the New World). However, incidental transmission by blood transfusions or by needle sharing among intravenous drug addicts has also been described [144, 145]. There are two main clinical presentations of the disease, cutaneous or mucocutaneous leishmaniasis (CL) and visceral leishmaniasis (VL), also known as Kala azar.

Based on recent estimates, approximately 0.2–0.4 million VL cases and 0.7–1.2 million CL cases occur each year [146]. There is growing evidence that the true incidence of the disease is underestimated, especially in hyperendemic regions [147]. Assuming a case-fatality rate of 10%, it is estimated that VL causes 20,000–40,000 per year. Mortality estimates have more uncertainty than incidence estimates as a large proportion of VL deaths occur outside of health facilities and the cause likely never recognized [146].

The disease is emerging in immunocompromised patients undergoing hematopoietic stem cell or solid organ transplantation or treatment with biologic drugs [16, 147, 148]. In transplanted patients, VL is the clinical presentation more frequently reported accounting for more than 85% of the leishmaniasis cases.

9.4.2.1 Geographic Distribution

In the Old World, CL are caused by *Leishmania tropica*, *L. major* and *L. aethiopica*, and VL are caused by the *Leishmania donovani* complex, which includes two species, *L. donovani* and *L. infantum*. In the Americas, various leish-

mania species are able to produce cutaneous leishmaniasis, such as: *L. braziliensis*, *L. amazonensis*, *L. guianensis, L. pananmensis* and *L. mexicana*. Visceral leishmaniasis in the New World is caused by *L. chagasi* [149].

According to a recent publication, a total of 98 countries and 3 territories on 5 continents reported endemic Leishmaniasis transmission. More than 90% of global VL cases occur in just six countries: India, Bangladesh, Sudan, South Sudan, Brazil, and Ethiopia [146].

The control of leishmaniasis is based in the control of the anthroponotic foci which are the source of severe and life-threatening VL epidemics. Dogs are the main source of infection and canine enzooty usually precedes the occurrence of cases in humans. Policies include early treatment of humans cases of VL, passive and active notification of canine leishmaniasis, vector control, use of screens in doors and windows, continuous education, and if necessary, humanitarian elimination of infected dogs.

The diagnosis of VL can be performed by microscopy of bone marrow aspirates, culture, serology and/or PCR. Microscopy of bone marrow aspirates is the preferred method with a sensitivity of more than 80%. Leishmania culture is laborious and subjected to contamination. Moreover, the low sensitivity in asymptomatic patients precludes its use as a screening test. Sensitivity of serology is variable and specificity can be unsatisfactory as it may present a cross-reaction with other protozoa [150]. PCR is the most effective test for the diagnosis and follow-up of VL, is less invasive than bone marrow aspirates, and is superior to serology to detect cases of infection [151]. In addition, PCR has been reported to be useful in the follow-up of treatment efficacy, also in immunocompromised patients [152]. Thus, PCR or the combination of techniques is regarded as preferred method in the diagnosis and follow-up of VL transplant recipients [148].

9.4.2.2 Transmission in Transplant Patients

According to a recent review, more than 100 cases of leishmaniasis have been described in organ transplant recipients [153]. Most of the cases have been described in renal transplant recipients, possibly reflecting the greater number of renal as compared to other organ transplants worldwide.

Leishmaniasis in transplant recipients may occur due to (1) reactivation of latent infection in a previously infected recipient during immunosuppression; (2) de novo infection in transplant recipients living in or traveling to areas of endemicity [154]; (3) transfusion-associated leishmaniasis, as routine serology for blood or organ donors is not performed even in areas of high endemicity [144]; (4) via an infected graft, as asymptomatic infection is more frequent than symptomatic disease even in endemic areas [148]. This route of transmission is more likely to occur in organs that form part of the reticuloendothelial system, such as liver. A study conducted in an endemic area of Brazil, showed that 23.5% of blood, 17.7% of liver and 6% of spleen samples from asymptomatic liver donors tested positive for *L. infantum* by PCR [150].

9.4.2.3 Pretransplant Management

Epidemiological risk. Evaluation of donor and recipient's epidemiological risk of leishmaniasis is required. Patients who live in or have travelled to endemic areas of the disease are at risk of developing disease. Spain, Brazil, France, and Italy have the highest number of reported cases [148, 153]. In countries like Brazil, the great number of reports just reflects local endemicity [155] whereas in European countries, VL cases have occurred in migrant transplant recipients or in SOT recipients from non-endemic areas who have travelled to an endemic region of leishmaniasis [156].

Screening: Pretransplant screening is not routinely recommended because the value of serologic or molecular screening of asymptomatic donors and recipients remains unclear.

9.4.2.4 Posttransplant Management

Risk factors and clinical findings: Clinical signs and symptoms of VL are prolonged fever, weight loss, hepatosplenomegaly, and pancytopenia. However, atypical forms, such as mucosal and cutaneous forms, have occasionally been reported [148]. The median time of VL occurrence is 25 months (range, 1–118). Serology, direct detection of leishmania amastigotes in bone marrow biopsy samples and PCR should be performed in suspected cases [148, 150, 151].

A recent collaborative case-control study performed in Spain and Brazil evaluated the risk factors, clinical features and outcomes of VL in SOT recipients. Thirty-six VL cases were identified among 25,139 SOT recipients (0.1%) with a crude mortality rate of 2.8% [157]. Parasites were identified in 89% of the patients; the remaining cases were diagnosed by serology. Fever, hepatosplenomegaly and pancytopenia were present in 86%, 81% and 47% of the patients, respectively. Patients were diagnosed over 1 month after symptom onset in 25% of cases. VL occurred 5.7-fold more frequently in Brazil than in Spain, presenting at a median time of 11 months after transplantation. Treatment with high-dose prednisone in the preceding 6 months was the only factor significantly associated with VL. Interestingly, induction therapy with antilymphocyte globulins or intensity of baseline immunosuppression was not related to the occurrence of VL. The same was observed in a recent publication of 30 cases of VL in renal transplant recipients [151].

VL monitoring: If a donor or a recipient is known to be positive, strict monitoring in the post-transplant period is advised not only for this potential life-threatening condition, but also for the potential role of VL in graft dysfunction and loss. Monitoring *Leishmania* DNA levels every 3–6 months for the first 24 months after an episode of VL is advisable, as

most of the recurrences occur in the first 2 years after the initial episode.

Treatment: Liposomal amphotericin is the drug of choice in the treatment of VL. Other drugs such as *N*-methyl-glucamine and miltefosine have been less frequently used. Side effects are the main obstacles to the use of pentavalent antimony. Miltefosine is an oral drug approved for the treatment of VL and for cutaneous leishmaniasis for more than one decade in some developing countries, such as India. Initial cure rates of more than 95% have been reported in nontransplant population, but an increase in the rate of VL relapses in patients treated with oral miltefosine is of concern [158]. Few information is available concerning to the use and efficacy of miltefosine in the setting of transplantation.

VL relapses: Transplant recipients also have an increased risk of disease recurrences, varying from 1 to 5 (median 1.7) relapses after treatment, occurring at a median of 13 months (range, 1–60) after the initial episode [159]. Frequency of VL relapses may vary but in general is around 25%. Two recent studies observed VL relapses in 25.7% and in 26.7% of solid organ and kidney transplant recipients, respectively [151, 157]. The use of secondary prophylaxis to avoid relapses did not show significant benefit in a retrospective case-control study. Relapse was observed in 1 (8.3%) of the 12 patients receiving prophylaxis and in 8 (34.8%) of the 23 patients not receiving prophylaxis ($p = 0.19$) [157].

Since no prospective studies are available, the real incidence, risk factors, morbidity and mortality of cutaneous and visceral leishmaniasis in different transplant populations, are unknown. So far, no strong recommendation can be made concerning to the use of secondary prophylaxis to avoid VL relapses.

9.4.3 Malaria

Malaria is an acute systemic illness caused by infection with *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, or *Plasmodium ovale,* which are the human malaria species. *P. falciparum* tends to be more virulent than other species. Globally, an estimated 3.3 billion people in 97 countries and territories are at risk of being infected with malaria. Since 2000, a decrease in malaria incidence and mortality rates of 30% and 47%, respectively has been observed. Still, 198 million cases of malaria occurred globally and the disease led to approximately 584,000 deaths in 2013 [160].

Malaria primarily affects low- and lower-middle income countries. Within endemic countries, the most severely affected are the poorest and most marginalized communities, which have the least access to appropriate prevention, diagnosis, and treatment. Thus, malaria control is intimately linked with health system strengthening, infrastructure development, and poverty reduction. The burden is heaviest in Africa, where an estimated 90% of all malaria deaths occur, and in children aged under 5 years, who account for 78% of all deaths [160].

Plasmodium species are transmitted to humans by female *Anopheles* mosquitoes. The life cycle of the parasites has two phases; an asexual replicative phase in humans and a phase of sexual replication in the mosquito. In the human body, the parasites multiply in the liver, and then infect red blood cells. The symptoms, signs, and pathological features of malaria are caused by the asexual erythrocytic stage of the parasites that invade and replicate in erythrocytes over 2–3 days, rupture these cells and reinvade normal erythrocytes. This exponential parasite replication in the bloodstream increases parasite density by 5 to 30-fold every 2–3 days. In the case of *P. vivax* and *P. ovale*, a proportion remains dormant (hypnozoites) inside the hepatocytes and these forms are responsible for late relapses [161].

9.4.3.1 Geographic Distribution

People in 97 countries around the world are at risk of malaria infection, mostly in Africa, Asia, and in Central and South America [160]. Awareness of *Plasmodium* species endemicity is important to early introduction of appropriate treatment. *Plasmodium falciparum* is found in sub-Saharan Africa, South-East Asia, and the Indian subcontinent as well as in South America, Haiti, the Dominican Republic, Jamaica and areas of Oceania. *P. malariae* and *P. ovale* are present in sub-Saharan Africa. *Plasmodium vivax* is prevalent in areas of South-East Asia, the Indian Subcontinent, and Central and South America [160, 161].

9.4.3.2 Transmission in Transplant Recipients

In transplant recipients, malaria may be transmitted through infected blood products, via an infected organ or by natural exposure to *Plasmodium*-infected mosquitoes in endemic regions. In transfusion-associated malaria, symptoms generally develop earlier (1–3 days) than if the infection is transmitted through infected cells within the organ (more than 1 week) [161].

Blood-borne transmission: Transfusion-transmitted (TT) malaria is severe and often fatal. The use of antibody tests in the prevention of TT-malaria varies worldwide. In a recent international forum evaluating the preventive measures taken in non-endemic areas, only 4 of the 14 participating countries perform malaria antibody tests routinely (United Kingdom, Denmark, Finland, and New Zealand). In Brazil, the Amazon region is considered as endemic, whereas the rest of the country is considered non-endemic. In non-endemic areas, testing is not required nor performed. Donors are prevented from donating for 6 months if they have been to endemic areas or 3 years if they had had malaria or lived in endemic area, and definitely rejected for *P. malariae* infection at any period of their lifetime. In endemic areas an index of parasit-

emia (IPA) is provided according to each municipality and district. This IPA allows donors to be classified into low-, medium- or high-risk. Donors from high-risk areas are rejected, whereas medium and low-risk are submitted to a predonation test that may be a rapid test (antigenic) or the thick smear. Positive donors are excluded from donating for 1 year and referred for treatment. After 1 year they are allowed to donate provided they present a negative parasitologic test [162].

Graft transmission: *Plasmodium* species are capable of surviving more than 24 h in blood at 4 °C. Therefore, the time of cold preservation is generally not enough to prevent transmission, especially in heart and liver transplantation that requires shorter cold preservation time: 3–4 h for heart and less than 12 h for liver, in comparison to 24–48 h for kidney. The reports of malaria cases following multi-organ donation have supported the hypothesis of the graft as a source of *Plasmodium* in transplant recipients [163–167]. Malaria deaths in SOT recipients are generally associates with *P. falciparum* infection. In HSCT recipients, past geographic exposure of both donor and recipients seems to be the main source of *Plasmodium* infection, even though transmission by blood transfusion has also been reported [166, 168]. In most cases, the diagnosis was made by the identification of the parasite in blood smears of febrile patients with unexplained hemolysis and thrombocytopenia.

Persistent malaria is the greatest challenge in the pretransplant screening or blood transfusion policies. Donors or transplant candidates, who were born or have lived in an endemic area, harbor the highest risk of malaria infection. Those individuals generally have high levels of infection and frequent exposure to malaria parasites, leading to a dynamic balance between the infection and the immune response to it. Such individuals are categorized as "semi-immune," as they are asymptomatic, have high-titer antibody and the majority have a resolved infection. However, a small number may still be infected with a persistent low-level parasitemia [169]. We recently report a case stressing the difficulty in eradicating *P. vivax* parasitemia from a semi-immune HSCT donor who had nine episodes of malaria during his lifetime [170].

Clinicians caring for transplant recipients in developing countries of known endemicity should take into account that the parasite may persist in humans who survived to untreated malaria. In case of semi-immune transplant recipients, posttransplant immunosuppression disrupts the balance between persistent infection and immune response and a bout of parasitemia may occur. Persistence is estimated to be 1 year for *P. falciparum,* 3–5 years for *P. vivax* and *ovale* and as long as 40 years for *P. malariae* [170]. Some reports have described persistent *P. falciparum* malaria in immigrants currently living in non-endemic areas for more than 9 years [171, 172].

9.4.3.3 Pretransplant Management

Epidemiological risk: Investigation of the epidemiological risk for malaria is mandatory for both donors and recipients. In developing countries, a past history of malaria is not a contraindication for organ or stem cell donation. Donors who have traveled to or lived in endemic areas should be deferred from donation for 6 months and 3 years, respectively. Of the only donor available, empirical treatment before donation should be considered.

Diagnosis: Identification of *Plasmodium* species is important to guide treatment. If possible, serology, rapid antigen tests and/or blood smear examination should be considered for all patients and donors at risk. Thick and thin blood film microscopy examination remains the gold standard for diagnosis, but specific antibody-based rapid diagnostic tests that detect PfHRP2, pan-malaria or species-specific lactate dehydrogenase, or aldolase antigens in fingerpick blood are now used widely [173]. Because of their simplicity and speed, rapid diagnostic tests are particularly valuable in epidemic investigations and surveys. However, they are expensive and do not quantify parasitemia. Molecular methods such as PCR have been used mainly as a research tool [170].

9.4.3.4 Posttransplant Management

Clinical findings: Clinicians must be aware that malaria not always has the typical paroxysmal or cyclic pattern in transplant recipients and a high index of suspicion should be maintained when caring for patients with identifiable risk factors. Fever, anemia, and neurological alterations are frequent findings of malaria in transplant recipients. Mortality rates vary from 10 to 40%. Malaria is more harmful in naïve recipients from non-endemic areas. The prognosis of posttransplant malaria depends on the delay in diagnosis and initiation of treatment, the species of *Plasmodium* involved, the type of organ transplanted, and the type of immunosuppressive therapy. The majority of deaths are associated to *P. falciparum*. Especial attention should be paid to splenectomized patients who may develop more severe disease because the spleen is responsible for the removal of parasitized cells from the circulation.

Treatment: *P. falciparum malaria* is treated with artemisinin-based combined therapy (artemether 20 mg) and lumefantrine (120 mg), piperaquine (160 mg) or mefloquine (1250 mg). Mefloquine is still used in areas of susceptible *P. falciparum. P. vivax* and *P. ovale* are treated with chloroquine (25 mg/kg for 3 days), which is still used in some countries in the Region of the Americas and primaquine (0.5 mg/kg/day for ≥7 days), currently the only drug available to treat the liver stage (hypnozoite) of *P. vivax* infection [160]. If donors or recipients have history of mosquito exposure in regions of *Plasmodium vivax* or *P. ovale*, clinicians should have in mind that the parasites' exoerythrocytic schizogony in the liver makes eradication more difficult. The dormant hypnozoites forms can cause relapse up to 12 months later. Primaquine is not necessary in blood or graft-transmitted malaria, as hepatic hypnozoites are not established in these cases. Mefloquine, doxycycline, chloroquine, and primaquine may

increase calcineurin inhibitors levels. G6PD deficiency should be investigated before the use of primaquine to avoid hemolysis [160].

In conclusion, we are living in an increasingly globalized world in which transmission of infectious diseases has no boundaries. Tourism travels, international commerce, and immigration have acted as important factors for the emergence and re-emergence of specific infectious diseases. An important proportion of migrant population will naturally become part of the working force as well as part of blood bank, transplant donor, or transplant recipient population in destination countries.

Many of the pathogens that cause tropical diseases can be either transmitted via graft or blood transfusions or reactivated during immunosuppression. Healthcare workers in the field of transplantation need to be prepared to recognize the epidemiological risks and to manage tropical infections in transplant recipients.

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10 Risks and Epidemiology of Infections Associated with Ventricular Assist Devices and Heart Transplantation

Amanda R. Vest, David DeNofrio, and David R. Snydman

The infectious complications of heart transplantation are similar to that seen in other solid organ transplants with respect to risk factors and timing. However, there are a number of unique aspects to the types of infections that occur in heart transplantation, by virtue of the location and type of surgery necessary to replace the organ, as well as the fact that cardiac muscle can carry certain unique pathogens, such as *Toxoplasma gondii* and *Trypanosoma cruzi*. In addition, the increasing utilization of ventricular assist devices (VADs) prior to transplantation provides its own unique challenges and often complicates the post-transplant course. Finally, the immunologic effects of viral infections such as cytomegalovirus (CMV) as well as other viruses can have profound impact on the coronary vascular system, contributing to allograft vasculopathy as well as acute and chronic rejection.

Infectious complications are very common in cardiac transplantation. In an analysis of 620 consecutive patients followed over a 16-year period at Stanford, approximately 1.67 infections occurred per patient to [1]. Infection was second to rejection as a cause of early death, but was the most common cause of late death. Among the episodes of infection, bacteria caused 43.6%, viruses 41.7%, fungi 10.2%, *Pneumocystis jiroveci* 4%, and protozoa 0.6%. Over the course of the study period, the authors noted marked improvements in outcome due to the prophylactic use of antivirals, antibacterials, and antifungals with a reduction of many of these infectious complications. This chapter will review those aspects of infection that are unique to cardiac transplantation. Donor screening, vaccination, and prophylaxis will generally be the same as that recommended in other solid organ transplant recipients. However, there are some unique aspects to donor screening cardiac transplantation that will be discussed in this chapter. The reader is referred to other chapters as appropriate for specific details as they pertain to general concepts of screening and prevention.

10.1 Continuous-Flow Left Ventricular Assist Devices and Total Artificial Hearts

Approximately 40% of patients in the United States undergo heart transplantation while supported with a surgically implanted VAD and therefore, an understanding of the infectious complications and management of such devices is essential. Heart failure affects approximately five million people in the United States and remains a highly fatal disease [2–4]. Despite improvements in medical therapy, it is estimated that over 250,000 people have refractory end-stage systolic heart failure [3–6].

The "gold standard" management of refractory end-stage heart failure is heart transplantation, but this intervention is limited by donor organ availability, limited to around 2400 transplants in North America annually [7]. In contrast, durable mechanical circulatory support utilization has risen exponentially over the past decade, with 3472 continuous-flow left ventricular assist device (LVAD) implants reported to the Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) in 2013 [8, 9]. The inflow LVAD cannula is implanted into the apex of the left ventricle, through which blood is drawn into pump, and then delivered to the aorta via and outflow graft that anastomoses to the proximal aorta (Figure 10-1a). The driveline is a percutaneous conduit that carries electrical wires from the pump, through the abdominal cavity and subcutaneous tissues to the skin and then outside the body to the LVAD system controller worn externally. In recent clinical trials, LVADs have been demonstrated to both control heart failure symptoms and improve survival when placed either as a "bridge to transplantation" (BTT) or as "destination therapy" (DT) for individuals who are not transplant eligible. Major challenges to the success of LVADs are device infection, pump thrombosis, strokes, and gastrointestinal bleeding [8–10].

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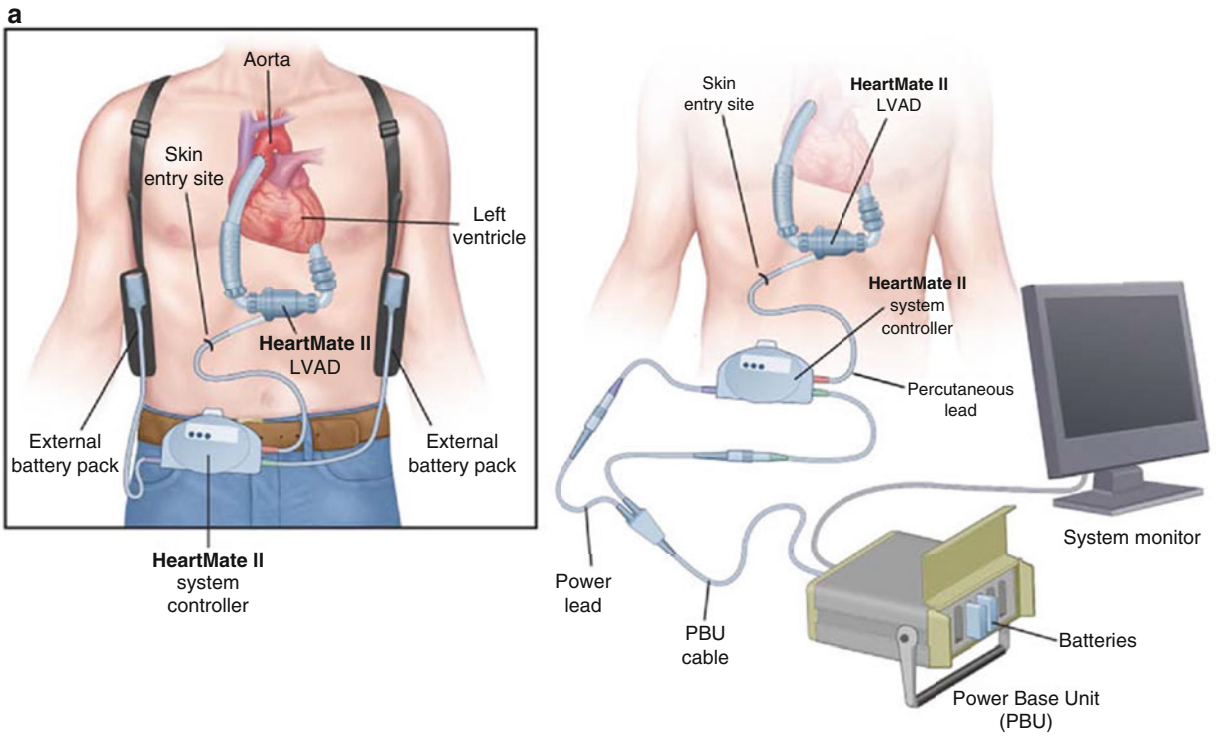

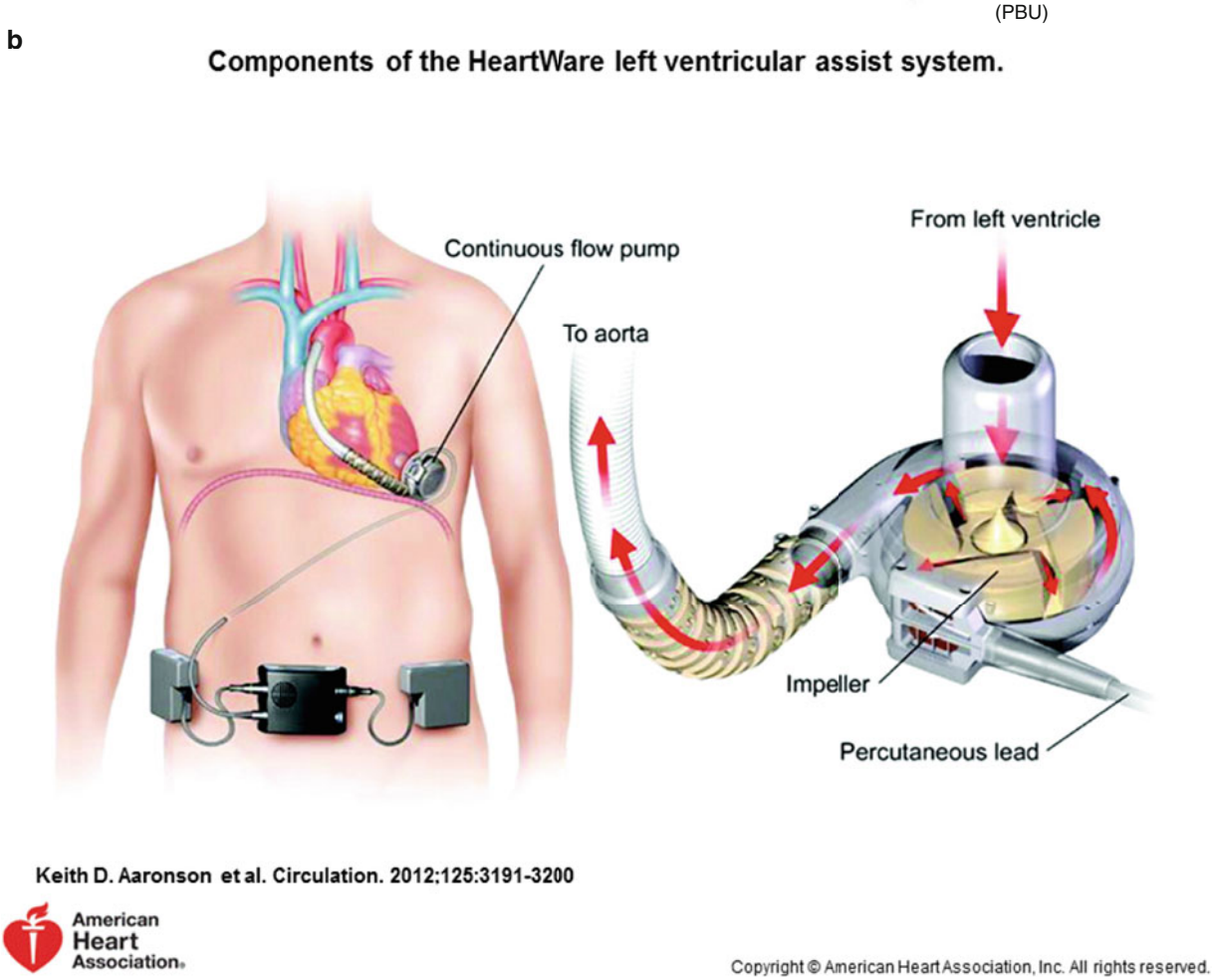

Keith D. Aaronson et al. Circulation. 2012;125:3191-3200

American Heart Association.

FIGURE 10-1. (**a**) Components of the HeartMate II left ventricular assist device system. Reprinted from Journal of the American College of Cardiology, 54(18), Wilson SR, Givertz MM, Stewart GC, Mudge GH, Ventricular Assist Devices: The Challenges of Outpatient Management, 1647–59, Copyright 2009, with permission of Elsevier; (**b**) components of the HeartWare left ventricular assist device system. Reprinted from Circulation, 125 (25), Aaronson KD, Slaughter MS, Miller LW, McGee EC, Cotts WG, Acker MA, et al., Use of an intrapericardial, continuous-flow, centrifugal pump in patients awaiting heart transplantation, 3191–200, Copyright 2012.

Although the rates of these complications are overall much lower in the current era of continuous-flow LVADs, as compared to original pulsatile devices, they remain a significant limitation to fully realizing the potential of this therapy.

The first generations of LVADs were pulsatile and were associated with large diameter drivelines, large abdominal pockets, and limited long-term mechanical durability. These devices have been superseded by continuous-flow technology. In 2007, a non-randomized study was published of 133 patients with end-stage heart failure awaiting heart transplantation who received a continuous-flow HeartMate II LVAD (Thoratec Corporation, Pleasanton, CA) [11]. This high-speed axial-flow, rotary pump was significantly smaller and lighter than the prior pulsatile pumps, with a reduced driveline diameter. At 180 days, 100 patients (75%) reached the principle outcome of heart transplantation, cardiac recovery, or survival with ongoing mechanical support and eligibility for transplantation. The survival rate was 68% at 12 months. At 3 months, there were significant improvements in New York Heart Association functional class, 6-min walk test results, the Minnesota Living with Heart Failure and Kansas City Cardiomyopathy questionnaires. Subsequently, the HeartMate II LVAD was FDA approved in April 2008 for use as durable BTT support [12]. The HeartMate II investigators also demonstrated the superiority of continuous-flow LVADs over pulsatile devices, in a DT cohort who were ineligible for heart transplantation [13]. The HeartMate II patients had superior actuarial survival rates at 2 years (58% vs. 24% in the pulsatile LVAD group, $p=0.008$). Mechanical failure, valve malfunction, infection, and the need for pump exchange were less frequent in patients receiving the continuous-flow device. Furthermore, there were significant improvements in quality of life and functional capacity in both groups. The extended results from the initial BTT trial were also published in 2009, with 222 of 281 patients (79%) reaching the endpoint of transplantation, LVAD removal for cardiac recovery, or ongoing LVAD support at 18-month follow-up [14]. This extended HeartMate II LVAD BTT study reported localized non-device-related infection in 30% of patients, sepsis in 17%, driveline infection in 14%, and pump pocket infection in 2%.

Subsequent continuous-flow LVAD publications demonstrated increasingly encouraging survival and infection rates. The HeartWare HVAD (HeartWare Inc., Framingham, MA) BTT trial studied a newer, smaller intra-pericardial continuous-flow LVAD with a centrifugal mechanical pump (Figure 10-1b) [15]. This was a non-randomized trial comparing HVAD recipients to a contemporaneous cohort of HeartMate II LVAD patients. A total of 140 patients received the HVAD pump, and 499 patients received a commercially available LVAD. Success, defined as survival on the originally implanted device, transplantation, or removal of the device for ventricular recovery at 180 days, occurred in 90.7% of HVAD recipients and 90.1% of HeartMate II LVAD controls, establishing non-inferiority of the investigational pump ($p<0.001$). At 6 months, median 6-min walk distance improved by 128.5 m, and both disease-specific and global quality-of-life scores improved significantly. Sepsis and driveline infections occurred in 11.4% and 12.1% of HVAD recipients, respectively. More recently, the HVAD has demonstrated non-inferiority to the HeartMate II LVAD for DT therapy in a preliminary report of the ENDURANCE trial [16]. Approximately 40% of LVADs are now implanted with a DT strategy, which brings with it an older and more comorbid group of patients who are more prone to infectious complications during support [8]. The high infection rate in the DT LVAD population of patients emphasizes the need for further studies directed toward effective antibiotic prophylaxis protocols prior to, at the time of, and following the LVAD procedure. Unlike the BTT population, the permanent therapy population does not have the opportunity to have the infected device explanted at the time of transplant, thus removing the source of infection and allowing adequate treatment of the infection. However, overall survival in continuous-flow LVAD recipients continues to improve, with the Sixth INTERMACS Annual Report describing actuarial survival among patients with continuous-flow pumps of 80% at 1 year and 70% at 2 years; DT survival exceeds 75% at 1 year and 50% at 3 years [8].

In patients who suffer refractory biventricular failure, it is sometimes necessary to mechanically support the right ventricle in addition to the left ventricle. This strategy is only pursued for BTT, not DT, patients. Depending on the clinical scenario and implanting center experience, the patient may receive a durable implantable right ventricular assist device (RVAD) in addition to their LVAD (thus requiring two driveline exit sites) or alternatively may undergo cardiac explanation and implantation of a total artificial heart (TAH). Patients requiring biventricular support (BiVAD) currently have inferior survival to LVAD recipients, with just under half of BiVAD patients alive at 12 months in the Sixth INTERMACS Annual Report [8]. Initially, all TAH recipients were confined to hospital until transplantation; however, with the introduction of a portable driver system some centers have successfully transitioned patients to home on the SynCardia TAH (SynCardia Systems Inc., Tuscan, AZ) [17]. One report of 47 recipients from centers across the world described systemic infection requiring intravenous antibiotics in 25 patients (53%). Driveline infections occurred in 13 patients (27%); in 2 of these patients (4%) the infection ascended into the mediastinum resulting in death. Five patients died of sepsis with multiorgan failure. There was an association between smaller body surface area and increased risk of infectious complications [18].

10.2 Left Ventricular Assist Device Infections

Infection remains a frequent complication in patients receiving continuous-flow LVAD support with incidence estimates ranging from 10 to 50% depending on the study cohort, time

frame, and diagnostic classifications. For example, the Sixth INTERMACS Annual Report describes 9.96 device infections per 100 patients-months in the first 12 months after continuous-flow LVAD or BiVAD surgery [8]. The extended HeartMate II LVAD BTT study reported localized non-device-related infection in 30%, sepsis in 17%, driveline infection in 14%, and pump pocket infection in 2% of patients enrolled. In the HeartWare HVAD BTT trial sepsis occurred in 11.4% and driveline exit site infections in 12.1% of recipients, which equates to 0.24 and 0.29 events per-patient-years, respectively [14, 15]. A single center study of 81 LVAD recipients reported that 51.9% of the cohort had at least one type of infection during continuous-flow LVAD support with a mean follow-up period of 9.2 months [19].

In the above report (Topkara et al.), patients with positive blood cultures, but no signs of systemic inflammatory response syndrome were categorized under systemic infections as a separate entity from sepsis. Local infections (non-LVAD related) were defined as those limited to any organ system or region without evidence of systemic involvement that requires treatment or is ascertained by standard clinical method. The LVAD-related infections (driveline or pump pocket) were defined as those that required treatment with antimicrobial therapy, when there is clinical evidence of infection such as pain, fever, drainage, and/or leukocytosis (Figure 10-2). Subgroup analysis revealed that post-implantation sepsis was significantly associated with increased mortality in the continuous-flow LVAD cohort (61.9% vs. 18.0% at 2 years, respectively, in septic and non-septic patients, $p < 0.001$). Resistant *Staphylococcus* and *Pseudomonas* species were the most common pathogens leading to device- and non-device-related local infections; however, development of driveline or pocket infection had no effect on survival in patients with continuous-flow assist device support ($p = 0.193$).

Sepsis especially early post-implant, VAD-related infective endocarditis and mediastinitis can have devastating consequences and have been associated with mortality rates as high as 70% [20]. Conversely localized driveline infections are often of limited clinical consequence if diagnosed and treated appropriately with local wound care and antibiotics. Infections in the abdominal wall pocket holding the device (for HeartMate II LVAD) or surrounding the pericardial location (for both HeartMate II LVAD and Heartware HVAD) require more aggressive treatment, including open drainage, debridement, and rerouting of the driveline through a fresh exit site. There is also limited experience with the implantation of antibiotic-coated beads to aid in control of the subcutaneous infection [21]. So long a device-related infection is controlled, it is not a contraindication to transplantation [22], and is often an indication for United Network Organ Sharing (UNOS) 1A transplantation status listing by exception. However, all device-related infections require careful review for appropriate ongoing management at the time of transplantation.

The International Society of Heart and Lung Transplantation (ISHLT) does not specify which antimicrobials should be administered as prophylaxis, but give class I recommendations for broad-spectrum Gram-positive and Gram-negative coverage, with at least one dose prior to surgery administered within 60 min of the first incision, and maintained in the therapeutic range throughout the duration of their use but not extended beyond 24–48 h (Table 10-1). They also support topical treatment for methicillin-resistant *Staphylococcus*

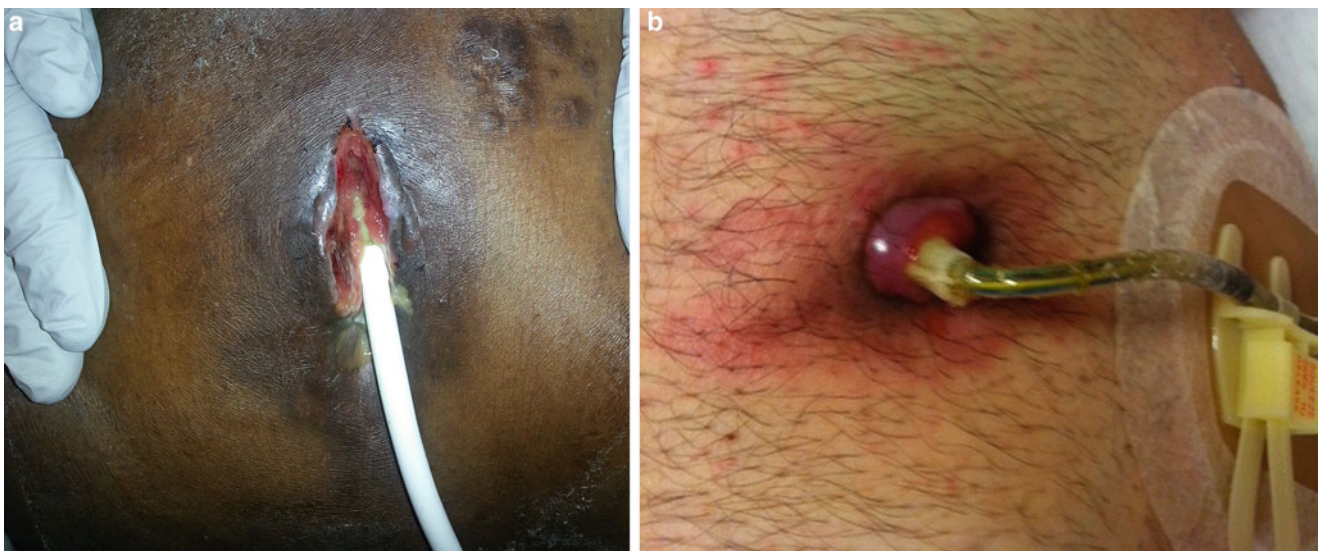

FIGURE 10-2. LVAD driveline infections. Photos of driveline infections in a patient with a HMII device (**a**) and a HVAD device (**b**), respectively.

TABLE 10-1. Recommendations for antimicrobial prophylaxis regimen from the 2013 International Society of Heart and Lung Transplantation Guidelines

Class I
1. Patients should receive preoperative antibiotics with broad-spectrum Gram-positive and Gram-negative coverage, as appropriate, prior to durable mechanical circulatory support implantation
Level of evidence: C
2. Routine antibiotic prophylaxis should include at least one dose prior to surgery administered within 60 min of the first incision, remain in the therapeutic range throughout the duration of their use, and not extend beyond 24–48 h
Level of evidence: C
3. Patients should have a nasal swab to screen for methicillin-resistant *Staphylococcus aureus and* receive topical treatment if positive prior to durable mechanical circulatory support implantation
Level of evidence: C

Reprinted from J Heart Lung Transplant, 32(2), Feldman D, Pamboukian SV, Teuteberg JJ, Birks E, Lietz K, Moore SA, et al., The 2013 International Society for Heart and Lung Transplantation Guidelines for mechanical circulatory support: Executive summary, 157–87, Copyright 2013, with permission of Elsevier.

aureus if a preoperative nasal swab is positive [23]. Other perioperative prevention strategies include making the driveline tunnel as long as possible. Once the lead has been tunneled in place, it is currently recommended that the Dacron-velour, which stimulates subcutaneous growth and sealing of the skin, is kept in the subcutaneous space so that only silicone is in contact with skin at exit site [23].

An ISHLT Infectious Diseases Working Group has sought to standardize the definitions and criteria for "VAD-specific infections," "VAD-related infections," and "non-VAD infections" (Table 10-2) [24]. Many studies have failed to elucidate risk factors for the development of LVAD infections. Obesity is one feature that may increase infection risks per a single-center report [25]. Patients who developed device-related infections had a significantly higher body mass index compared with the control group and continued weight gain over the course of LVAD therapy. Another potential contributor, although difficult to quantify, is driveline trauma, which is thought to contribute to infection onset due to loss of tissue in-growth at the exit site [26]. Patient and caregiver attention to the driveline site is clearly an important factor, although again difficult to quantify. Interestingly, one perspective multicenter study found that a history of depression was the strongest independent predictors of VAD infection (adjusted hazard ratio = 2.8, 95% CI 1.3–6.0, $p = 0.007$) [27].

Although bacterial infections predominate, fungal VAD infections are certainly not insignificant and carry a high morbidity and mortality rate. One center followed 300 VAD patients of which 108 developed a device-related infection; 85 were bacterial and 23 were fungal [28]. The most common bacteria were *Staphylococcus aureus*, coagulase-negative staphylococci, enterococci, and *Pseudomonas aeruginosa*. The most common fungal infection was *Candida albicans*. Only the use of total parenteral nutrition was associated with the development of a fungal VAD infection in multivariate analysis (odds ratio, 6.95; 95% confidence interval, 1.71–28.16, $p = 0.007$). Patients who experienced fungal VAD infection were less likely to be cured (17.4% vs. 56.3%, $p = 0.001$) and had greater mortality (91% vs. 61%, $p = 0.006$), compared with those who experienced bacterial VAD infection.

Recipients of LVADs are prone not only to device-related infection, but also to nosocomial infections, of which is commonest are respiratory tract infections [29]. The former occur as a result of the percutaneous driveline exiting the skin allowing the entrance of bacteria, LVAD pocket bacterial seeding at the time of surgery, or tracking of bacteria from the driveline site, and endovascular infections of the blood-contact device components that become seeded from transient bacteremia [30]. It is hoped that a future totally implantable VAD system that does not require a percutaneous driveline may markedly reduce rates of such infections. Nosocomial infections occur as a result of the patient's prolonged hospitalization, suboptimal nutritional status, immobilization, endotracheal intubation, and need for multiple intravascular and bladder catheters. Furthermore, there may be LVAD-associated defects in cellular and humoral immunity, including selective reduction in the number of $CD4^+$ T cells, defective proliferative responses of peripheral mononuclear cells, increased apoptosis of $CD4^+$ and $CD8^+$ T cells and B-cell hyperreactivity. However, these potential mechanisms of immunologic susceptibility to infection were identified in the pulsatile VAD era and may not be contributors in the setting on continuous-flow support [31–34].

10.3 Left Ventricular Assist Device Effect on Post-transplant Outcomes

There is conflicting data regarding the impact of LVAD bridging on subsequent post-transplant outcomes. Early reports included a Cardiac Transplant Research Database (CTRD) review of over 5880 patients transplanted between 1990 and 1997, comparing 502 patients receiving pulsatile LVAD support to 2514 patients receiving only intravenous inotropic support at the time of heart transplantation [35]. Survival between the two groups was comparable. The most frequent post-transplant causes of death in the LVAD patients were infection (27%), followed by early graft failure (18%) and acute rejection (10%). Interestingly in this analysis, the LVAD patients had a significantly lower overall freedom from first infection compared with medical therapy patients. Other studies from this era had similar findings, including that device-related infection was unassociated with post-transplant mortality [36, 37].

Table 10-2. Definitions of ventricular assist device-specific percutaneous driveline infection

	Surgical/histology	Microbiology	Clinical	General wound appearance
A. Superficial VAD-specific percutaneous driveline infection				
Proven—Surgical/histology criteria ± other criteria	• Involvement of tissues superficial to the fascia and muscle layers of the incision documented	• Aseptic skin culture positive or not cultured	• Local increase in temperature around the exit site	• Purulent discharge from the incision but not involving fascia or muscle layers or • Erythema spreading around the exit site[a]
Probable—No surgical/histology criteria with purulent discharge ± other criteria	• Surgical debridement not performed • No histology	• Aseptic skin culture positive or negative but patient already on antibiotic or had antiseptic used to clean wound	• Local increase in temperature around the exit site and • Treated as superficial infection with clinical response	• Purulent discharge from the incision but not involving fascia or muscle layers or • Erythema spreading around the exit site[a]
Possible—No surgical/histology or purulent discharge ± other criteria	• Surgical debridement not performed • No histology	• Aseptic skin culture positive or negative and patient not on antibiotics or had antiseptic used to clean the wound	• Local increase in temperature around the exit site and • Treated as superficial infection with clinical response	• No discharge • Erythema spreading around the exit site[a]
B. Deep VAD-specific percutaneous driveline infection				
Proven—Surgical/histology criteria ± other criteria	• Involves deep soft tissue (e.g., fascial and muscle layers) on direct examination or on direct examination during reoperation	• Culture positive or histology puncture positive for infection	• Temperature >38 °C	• A deep incision spontaneous dehiscence
	• An abscess is found on direct examination during reoperation		• Localized pain or tenderness	• Abscess deep to the incision around the driveline
Probable—No surgical/histology criteria with spontaneous dehiscence ± other criteria	• No surgical debridement • No histology	• Culture negative but patients already on antibiotics or had antiseptic used on exit site	• Temperature >38 °C or • Localized pain or tenderness and • Treated as a deep infection	• An incision spontaneous dehiscence
Possible—No surgical/histology criteria with positive ultrasound ± other clinical criteria	• No surgical debridement • No histology	• Cultures not reserved	• Localized pain or tenderness and • Treated as a deep infection with clinical response	• Positive ultrasound

VAD ventricular assist device.

Reprinted from J Heart Lung Transplant, 30(4), Hannan MM, Husain S, Mattner F, Danziger-Isakov L, Drew RJ, Corey GR, et al., Working formulation for the standardization of definitions of infections in patients using ventricular assist devices, 375–84, Copyright 2011, with permission from Elsevier.

[a]Erythema excluding stitch abscess (minimal inflammation and discharge confined to the points of suture penetration).

However, UNOS data on patients transplanted between 1995 and 2004 has a small excess in mortality in the first 6 months (hazard ratio 1.20, 95% confidence interval 1.02–1.43) and then also beyond 5 years post-transplant for those bridged by pulsatile LVADs [38]. In contrast to prior reports, infection appeared to be part of the reason for excess mortality during the early post-transplant period in the LVAD patients. The risk of infection-related mortality in the first 12 months following transplant was increased for patients with an LVAD (HR [95% CI], 1.41 [0.98–2.03]) compared to non-LVAD patients (Table 10-3). Similar results were obtained by a 2006–2012 UNOS data analysis focusing on continuous-flow LVAD patients who were UNOS status 1A at the time of transplantation [39]. A total of 2113 LVAD-supported status 1A transplant candidates were divided based on the presence (45%) or absence (55%) of device-related complications (DRCs). Device-related infection was the stated DRC in 513 patients (54% of DRC group). During the study period, the prevalence of Status 1A patients supported with continuous-flow LVADs whose listing status was attributable to DRCs increased from 20% in 2006 to 55% in 2012. Although all types of DRC increased over time, most of the increase was noted in the device-related infections category. Post-transplant survival was inferior for the DRC group

TABLE 10-3. Comparison of specific causes of death among patients with VAD vs. those within the first 12 months after transplant

	Number of deaths			Hazard ratio (95% CI)[a]	
Cause of death	UNOS status 1 ($n=9455$)	Intracorporeal VAD ($n=1433$)	Extracorporeal VAD ($n=448$)	Intracorporeal VAD	Extracorporeal VAD
Rejection	173	21	10	0.91 (0.54–1.55)	1.34 (0.67–2.69)
Infection	276	49	23	1.41 (0.98–2.03)	2.17 (1.35–3.46)
Cardiovascular	246	33	19	0.89 (0.59–1.35)	1.55 (0.93–2.58)
Pulmonary	75	7	5	0.60 (0.25–1.41)	1.48 (0.56–3.91)
Malignancies	23	3	1	1.17 (0.28–4.83)	2.36 (0.28–19.96)
Other	568	104	54	1.07 (0.83–1.37)	1.66 (1.21–2.26)

Reprinted from J Am Coll Cardiol, 53, Patolla V, Patten RD, DeNofrio D, et al. The effect of ventricular assist devices on post-transplant mortality: An analysis of the United Network for Organ Sharing Thoracic Registry, 264–271, Copyright 2009, with permission of Elsevier.
[a]Compared to other UNOS Status 1 patients.

compared to those without DRCs at 1 year (85.6% vs. 89.9%, $p=0.0143$) and at 3 years (77.9% vs. 82.7%, $p=0.0116$). Of the five categories of DRCs, survival was lower than in the non-DRC group only for the device-related infections category, both at 1 year (85% vs. 89.9%) and at 3 years (77% vs. 82.7%, $p=0.01$). This small decrement in early postoperative survival associated with LVAD infections may be related to the frequency of pre-transplant device infections resulting from impaired cellular immunity described in LVAD patients [30]. An alternative explanation could be that the sicker transplant candidates require more inpatient and outpatient hospital visits and are at increased of contracting and/or being diagnosed with a device-related infection. It is important to note that the same bacteria isolated pre-transplant may be found as a pathogen following transplantation and prophylaxis or treatment should be directed at the previously identified pathogen.

10.4 Anatomic and Surgical-Related Infections

Post-transplant mediastinitis is a potential complication of heart transplantation, occurring in approximately 5–10% of patients. Orthotopic heart transplant recipients have a higher risk of mediastinitis compared to other cardiothoracic procedures. Factors associated with an increased risk of mediastinitis include prior heart transplant, UNOS status 1 designation (the sickest patients), diabetes mellitus, reoperation for mediastinal bleeding, and coronary artery disease in addition to heart failure. In addition, the presence of *S. aureus* pneumonia postoperatively, or the need for treatment for rejection increase the risk [40, 41]. VAD infections that precede transplantation may also be a predisposing condition. One retrospective study of deep sternal wound infections found that body mass index over 30 kg/m^2, previous heart surgery, and previous VAD were associated with infection [41]. In-hospital mortality was increased in patients with this complication. If one can successfully treat the deep sternal wound infection, the long-term prognosis is comparable to patients who do not suffer this complication [42].

In a prospective observational study by the Spanish National Hospital Network (RESITRA) of 282 heart transplant recipients, 4.8% developed incisional surgical site infections, of which only one developed mediastinitis [43]. The median time from transplant to incisional site infection was 14 days (range 3–75 days). The organisms were mostly the patient's own flora, *S. aureus*, or coagulase-negative staphylococci predominate. But other pathogens can occur, including *Candida*. The prognosis with incisional site infections, other than mediastinitis, in the Spanish series was good.

Therapy of mediastinitis following heart transplantation is similar to that following other cardiac surgery. Debridement, a prolonged course of antibiotics, and potential use of muscle flaps or skin grafts may be necessary [42]. However, deep-seated wound infections can be devastating.

10.5 Other Bacterial Infections

Pneumonia is another common complication following heart transplantation. It may occur in up to 30% of patients [43–45]. In an analysis from the Mayo Clinic, the incidence was shown to decrease over three 5-year periods from 40 to 18%. Despite improvements in prevention of pneumonia, occurrence of pneumonia was independently predictive of mortality in heart transplant recipients.

Bacteremia following heart transplantation has been demonstrated to occur in approximately 16% of all heart transplant recipients [43]. Most common sources of bacteremia were lower respiratory (23%), urinary (20%), and intravascular catheter (16%). In this series from Spain, Gram-negative organisms were slightly more common than Gram positives. Risk factors for blood stream infection included the need for hemodialysis, prolonged intensive care requirements (defined as more than 5 days), and viral infections. Blood stream infection in heart transplant recipients was an

independent predictor of mortality (odds ratio of 1.8, with 95% confidence interval between 1.2 and 2.8). This increased risk of mortality from bacteremia has been demonstrated in other types of organ transplant recipients as well [46].

Endocarditis can occur following orthotopic heart transplantation. In one analysis, mortality was higher with a shorter median survival compared to patients who were not heart transplant recipients [47].

Among bacterial infections, the most common site of infection is the lung (35%), followed by the urinary tract (24%), blood stream (7.7%), and intra-abdominal (5.8%). *Escherichia coli* and *S. aureus* are the two most common pathogens, although *Pseudomonas*, *Klebsiella*, and *Enterobacter* species were quite common. The list of common bacterial pathogens generally follows what one might see as the list of common hospital acquired pathogens, reflecting the fact that most of the bacterial infections are nosocomially acquired.

Other less common bacterial infections, such as *Legionella*, *Listeria*, and *Nocardia*, which occur in immunocompromised hosts, are covered in more detail elsewhere (see Chaps. 20 and 23). In cardiac transplantation series, *Legionella* occurred in approximately 5% of the bacterial infectious episodes, as did *Nocardia* [48–51]. For *Legionella* and *Nocardia*, the lung was the most common site of involvement. *Listeria monocytogenes* occurred in approximately 2.5% of bacterial infectious episodes. There are no presentation or management issues that are unique to heart transplantation for these organisms.

Mycobacterial infection is a generally rare occurrence in cardiac transplantation in the United States [52]. Cases can occur at any time post-transplant. In one series only three cases of *Mycobacterium tuberculosis* were documented to occur in 620 transplant recipients. Atypical mycobacterial disease was more than twice as common, with eight cases being seen [53]. A recent observational cohort study of heart transplant recipients demonstrated that previous cardiac valve disease and chronic obstructive pulmonary disease was a risk factor for late infection, defined as that occurring more than 6 months post-transplant [54].

10.6 Fungal Infections Following Heart Transplantation

Aspergillus is the most significant fungus to affect cardiac transplant recipients [1, 55]. Among 109 fungal infections identified over a 20-year period (which did not include *P. jiroveci*), *Aspergillus* represented about 50%. The most common site was the lung, with disseminated disease occurring in 35% of cases. *Aspergillus* occurred most frequently in the first 3 months post-transplant. When dissemination does occur, the brain is often the most common site, and the presence of such an abscess or disseminated disease can be quite silent. *Aspergillus* is associated with the highest attributable mortality of any infection, and in one series disseminated disease had a mortality rate of 90%, and pulmonary disease of 40% [1]. In another series of *Aspergillus* infections post-cardiac transplantation, sepsis, multiorgan failure, and death were notable. Early onset cases (within 90 days of transplant) had traditional risk factors of hemodialysis, thoracic reoperation, and the presence of another case in the institution (reflecting perhaps a nosocomial problem). Later onset cases also included the need for hemodialysis as well as the need for augmented immunosuppression [56]. Targeted antifungal prophylaxis with caspofungin was shown to reduce invasive Aspergillosis in one outbreak among heart transplant recipients [57].

Candida infections are the second most common fungal infection in cardiac transplantation. In the Stanford series, about 33% of the fungal infections were due to *Candida*. Usually, the most common manifestation is mucosal infection of the oropharynx and in the Stanford experience this did represent one-third of all *Candida* infections. Other sites include dissemination, which can occur from esophagitis, or from an infection of an intravascular catheter, or from the driveline infection from the LVAD. The infections with *Candida* tend to occur in a more broadly distributed temporal pattern after the transplant. Often cases may be seen many months post-transplant. Most of these are mucosal in nature.

Coccidiomycosis and cryptococcosis occur rarely. They represent about 4% of the fungal infections seen at Stanford. In other series other endemic mycoses, such as histoplasmosis and blastomycosis, seem to affect cardiac transplant recipients at a rate similar to that seen in other types of solid organ transplantation [55].

P. jiroveci infection occurred in approximately 4% of all cardiac transplant recipients [1]. This complication has been reduced dramatically with the introduction of routine trimethoprim-sulfamethoxazole prophylaxis [58]. Cases still can occur many years post-transplant, and with any increase in immunosuppression, reactivation can occur.

Prevention strategies for fungal infections in heart transplantation are similar to other types of solid organ transplant recipients. Strategies include the use of mucosal prophylaxis such as nystatin swish and swallow, environmental controls, inhaled amphotericin B, or use of prophylactic systemic antifungals such as fluconazole, caspofungin or other echinocandins, or voriconazole. Since invasive fungal infection in cardiac transplantation is not as common as lung or liver transplants, routine use of systemic antifungal prophylaxis is not warranted. However, when patients are being treated for rejection, or have coexisting comorbidities, such as need for dialysis for renal failure, prophylaxis may be warranted. And as indicated above in an outbreak situation in which nosocomial cases of aspergillosis have arisen, some form of prophylaxis might be warranted. Routine use of trimethoprim-sulfamethoxazole for the first-year post-transplant is warranted, both for *P. jiroveci* prevention as

well as toxoplasmosis prevention (see antimicrobial prophylaxis). For a more detailed discussion of fungal infections, the reader is referred to Chaps. 39, 41 and 42.

10.7 Cytomegalovirus

Cardiac transplantation, like other forms of organ transplantation, carries with it a significant risk of CMV infection. CMV is the most important infectious factor that leads to a favorable or unfavorable outcome. In fact, the Stanford group described 20 years ago the impact of CMV infection on patient and graft survival [59]. The clinical risk factors for CMV in heart transplantation are identical to those seen in other forms of organ transplantation [60, 61]. The clinical presentation is also similar to that seen in other forms of organ transplantation with a couple of exceptions. One, CMV infection has been linked to coronary vasculopathy and accelerated atherosclerosis in heart transplant recipients, in contradistinction to the transplantation of other organs [59, 62]. The theory is that CMV can infect endothelial cells, and influence smooth muscle growth as well as migration, with proliferation within the coronary vasculature [63]. We also know that CMV infection upregulates proinflammatory cytokines, which leads to enhanced production of growth factors. There is also a procoagulant response which may also lead to vascular thrombosis [64]. This manifestation of the indirect effects of CMV is most pronounced in animal models and human studies. Two, there has been an association of CMV and left ventricular dysfunction as well [65]. There is evidence that effective CMV prevention strategies will actually reduce the incidence of intracoronary atherosclerosis, improve survival [66], as well as decrease the risk of opportunistic infections [67].

Another clinical feature of CMV in heart transplantation is the occurrence of myocarditis [68]. This is a relatively uncommon complication of CMV infection, but is generally seen only in and is unique to heart transplant recipients.

Prevention of CMV infection and disease in cardiac transplantation is similar to that recommended for other organs. Prophylaxis or preemptive therapy has been mainstay of prevention, depending upon program resources and patient population. Early studies with intravenous ganciclovir demonstrated that 1 month of intravenous ganciclovir dramatically improved the risk of CMV disease in all groups, but the CMV donor seropositive to recipient CMV seronegative mismatch [69]. Subsequent studies examining prophylaxis with oral ganciclovir as well as valganciclovir have demonstrated that CMV infection and disease can be prevented to a great extent in cardiac transplantation [70].

One difference in the prevention of CMV in cardiac transplantation compared to kidney or liver transplantation is the adjunctive use of CMV immune globulin, which is recommended by some authorities due to improvement in the degree or frequency of coronary vasculopathy in those receiving CMV immune globulin [71]. Limited data suggest that the use of CMV immune globulin with ganciclovir or valganciclovir is associated with an increase in the lumen size of coronary arteries as assessed by intracoronary ultrasound when compared to patients who just receive antiviral prophylaxis alone [67]. Further studies are needed to confirm these findings. In addition, there are cohort studies based on large patient registry sources of cardiac transplantation that show that antiviral CMV prophylaxis is associated with a decreased likelihood of graft loss [72] as well as improved survival [73]. A more complete discussion of CMV in solid organ transplantation is provided in Chap. 25. Regimens for CMV prophylaxis are outlined in Chap. 25.

Treatment of CMV disease, when it occurs in the heart transplant recipient, is the same as treatment given to other solid organ transplant recipients. The presentation of CMV disease, when it occurs, may be slightly different than that seen with other organs in that accelerated atherosclerosis, and even myocardial infarction may be a presenting sign or symptom of CMV infection. In addition, as mentioned earlier, myocarditis may occur as well, albeit rarely.

10.8 Other Viral Infections Following Heart Transplantation

Other viral infections in heart transplantation contribute to morbidity and mortality. It has been demonstrated that viral infections, including a variety of respiratory viruses contribute to the development of coronary vasculopathy and graft loss, presumably by upregulating inflammatory markers much like CMV [74]. One study of 553 endomyocardial biopsy specimens from 149 pediatric heart transplant recipients demonstrated the presence of viral genomes in the heart. Positive PCR for a viral genome was associated with an increase in cardiac adverse events over the ensuing months. Adenovirus was the most common viral genome detected [74].

Viral hepatitis can occur after heart transplantation, but transmission of hepatitis B is generally uncommon from the donor heart since donor screening to eliminate hepatitis B surface antigen (HBsAg) positivity is performed. Recipients with active hepatitis B carriage will reactivate in the setting of immunosuppression [75, 76]. Cardiac donors who are isolated anti-hepatitis B core (anti-HBc) positive can be used for heart transplantation with relatively little risk to the recipient [77]. Heart transplant recipients who are either hepatitis B or hepatitis C positive at the time of transplant tend to have a good prognosis, and their survival seems comparable to those without such infection according to several analyses [77, 78].

Post-transplant lymphoproliferative disease (PTLD), due to Epstein–Barr virus (EBV), has been reported to occur in about 3% of all heart transplant recipients, second to lung

transplant recipients in terms of risk [79, 80]. The exact reason for this increased risk is not entirely clear. Risk factors for PTLD in heart transplant recipients, namely primary EBV infection, as well as treatment with lymphocyte-depleting antibodies is similar to other types of organ transplants. Presentation of PTLD and treatment of PTLD does not differ from other forms of solid organ transplantation (see Chap. 24). It should be noted that the allograft may be involved when disease presents early, that is, less than 1-year post-transplant.

Human immunodeficiency virus (HIV) was previously considered an absolute contraindication to solid organ transplantation, but since the developed of anti-retroviral therapy there has been limited experience with heart transplantation in patients with HIV. Furthermore, there have been legal moves to prevent discrimination of potential transplant recipients based solely on HIV status [81]. An initial case report described a young male with prior opportunistic infections whose HIV viral load was undetectable prior to transplantation [82]. He had no opportunistic infections for the first 2 years post-transplant, but did have several asymptomatic cellular rejection episodes, anemia, thrombocytopenia, and fluctuations in his CD4 count. A single center subsequently reported 7 cases of HIV infection in heart transplant recipients; 5 were diagnosed with HIV before transplantation and 2 patients seroconverted after transplantation (at 1 and 7 years post-transplant) [83]. Five patients experienced asymptomatic rejection episodes. Cardiac allograft function remained normal in 6 patients. One patient who seroconverted to HIV-positive status 7 years after transplantation was diagnosed with cardiac allograft vasculopathy 5 years after transplant, resulting in ischemic cardiomyopathy. All patients were alive at a mean follow-up of 30 months (range, 3–88 months). The two patients who seroconverted to HIV-positive status after receiving transplant were initiated on anti-retroviral therapy. No AIDS-defining illness, low CD4 count, or detectable viral loads were documented in any patients post-transplant. A woman who seroconverted to HIV 1-year post-transplant also subsequently experienced cellular rejection, but was reported to have normal allograft function at 10 years post-transplant [84].

10.9 Parasitic Infections Following Heart Transplantation

A major distinction between heart transplant recipients and other organ transplants is the extent to which parasitic infection may occur due to transmission from the transplanted heart. *T. gondii* persists in cardiac muscle tissue and is thus transplanted via the donor organ. Thus, heart transplant recipients have the highest risk of toxoplasmosis. Among those who are mismatched serologically, that is, the toxoplasma antibody positive donor heart to the toxoplasma antibody negative recipients, 75% will develop primary toxoplasma infection in the absence of any intervention or prophylaxis [85, 86]. Among those who are seropositive for toxoplasma infection at the time of transplantation, reactivation can also occur [87]. The disease in cardiac transplants can be fatal disseminated disease, or myocarditis [88]. There has been some recent debate in the literature about the prognostic significance of toxoplasma serology on outcome following cardiac transplantation. Two reports suggested that being toxoplasma seronegative at the time of transplant was associated with a worse prognosis, but a subsequent study refuted those data in a larger cohort [89–91]. Prophylaxis with bactrim has fortunately reduced the risk of toxoplasmosis dramatically and is effective [1, 86]. Since it is used routinely for *P. jiroveci* prophylaxis, an added benefit is the prevention of toxoplasmosis. For more detailed discussion, the reader is referred to Chap. 44.

T. cruzi [92] is also quite important in heart transplantation since the parasite is dormant in cardiac tissue. Especially in Brazil and Argentina, a high proportion of organ donors will be seropositive for *T. cruzi*, and are capable of transmission [93]. Among those recipients with a donor who is seropositive for *T. cruzi*, about 20% will develop parasitemia within the first 6 months post-transplant [94]. In addition with immunosuppression reactivation may occur [95]. Intervention is possible with benznidazole. In addition, patients with *T. cruzi* cardiomyopathy may require transplantation. A recent analysis in the United States demonstrated a high number of patients having unrecognized Chagas cardiomyopathy having emigrated from endemic countries [96, 97]. Studies performed over the past two decades indicate that with proper management, that is, screening for parasitemia, and treatment with benznidazole 10 mg/kg/day for 60 days, mortality related to *T. cruzi* infection is very low and survival rates are comparable to heart transplants not infected with *T. cruzi* [97]. However, this agent is not available in the United States; only nifurtimox, a nitrofuran derivative is available through the Centers for Disease Control and Prevention. If patients do develop clinical Chagas disease, they may develop disseminated disease with myocarditis, subcutaneous nodules, and dissemination to other organs.

10.10 Hypogammaglobulinemia Following Heart Transplantation

Hypogammaglobulinemia has been shown to occur following cardiac and other organ transplantation [98, 99]. Severe hypogammaglobulinemia (defined as IgG levels < 350 mg/dL) occurred in 10% of heart transplant recipients followed prospectively at Cleveland Clinic [98]. Those with severe hypogammaglobulinemia were more likely to have treatment for more episodes of cellular rejection during the first 6 months post-transplant than those without such treatment. Severe hypogammaglobulinemia has been associated with an increased risk of bacterial and opportunistic fungal infec-

tions. In another study of heart transplant recipients, pre-transplant levels of IgG, below median values of 1140 mg/dL were shown to be associated with an increased risk of developing infections [100]. In addition, levels at 7 days post-transplant below the median value of 679 mg/dL were markedly associated with an increased risk for infection. These studies raise the question of the need for more intensive and routine monitoring of immunoglobulin levels.

Studies of replacement therapy with intravenous immune globulin, including CMV immune globulin, in the presence of severe deficiency have been shown to reduce the incidence of opportunistic infections and even rejection episodes in heart transplant recipients [101]. In cardiac transplant recipients it may be especially important to screen for the presence of hypogammaglobulinemia post-transplant to avoid such complications and to administer intravenous immune globulin to restore immunity and reduce the risk of infectious complications [101].

10.11 Antimicrobial Prophylaxis for Heart Transplantation

At the time of cardiac surgery, patients should receive antimicrobial surgical prophylaxis in accordance with Surgical Care Improvement Program (SCIP) guidelines. Forty-eight hours is all that is recommended. The use of trimethoprim-sulfamethoxazole, a single strength of 80 mg/400 mg per day for 6–12 months, or three times a week with the double strength, has been effective in reducing the risk of *P. jiroveci*, *T. gondii*, and even *Listeria monocytogenes* as well as *Nocardia*. The trimethoprim-sulfamethoxazole regimen has even been effective in preventing disseminated toxoplasmosis among the toxoplasma mismatched seropositive donor to seronegative recipient [102].

The recommendations for CMV prevention in general are outlined in Chap. 25. Among heart transplant recipients, prophylaxis with valganciclovir 900 mg orally once a day for 3 months is sufficient if one chooses this modality. For CMV seropositive donor to CMV seronegative mismatches, a recent study, which included heart transplant recipients, demonstrated that 6 months of prevention with valganciclovir is superior to 3 months, with a reduction in late onset disease [103]. Because of the potential immunomodulatory benefits of CMV immune globulin in cardiac transplantation, with reductions in allograft vasculopathy, some authorities recommend combining valganciclovir with CMV immune globulin for those patients who receive a CMV seropositive donor heart [72].

10.12 Immune Monitoring

There is an emerging body of literature that has looked at CD4 counts as well as other forms of immune monitoring. One analysis of T lymphocyte subsets suggests an independent association of opportunistic infections and CD4 nadir counts [104]. In another analysis from a program in Tel Aviv, the Immuknow assay (Cylex Inc., Columbia, MD) was used prospectively to follow patients at risk for rejection or infection [105]. The authors demonstrate a relationship between the assay, the development of opportunistic infection or rejection [105]. Subsequent follow-up of patients on everolimus confirm the utility of the assay for infection [106] but not rejection. If confirmed, assays such as these may become important to balance the level of immunosuppression to avoid both infection and rejection. Confirmatory studies with larger numbers of patients are clearly warranted but these data look promising. Another group has pursued the development of an immunologic score to reflect the level of immunosuppression. The score consists of an analysis of lymphocyte subsets at 1-week post-transplant along with immune globulin levels and complement levels [107]. They purport a very high hazard ratio for infection risk. These attempts to quantify levels of immune suppression and infection risk are promising, but require further analysis in large cohorts.

10.13 Summary

Infections in cardiac transplantation have declined over the past 30 years. The incidence of bacterial and fungal infections has declined quite dramatically, with rates dropping fivefold and sixfold, respectively. In addition, rates of CMV disease have also declined. Much of this is attributed to the routine use of prophylactic agents listed above as well as improvements in the surgical technique, intensive care unit management, and diagnosis of many of these infections.

The research agenda for the future includes the need for studies of optimal prevention and management of left ventricular assist device infections, including antibiotic prophylaxis. In addition, improvement in management of chronic graft rejection and vasculopathy are warranted.

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11
Risks and Epidemiology of Infections After Lung or Heart–Lung Transplantation

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11.1 Introduction

Currently, lung transplantation (LT) is an established therapeutic option for patients who have severe respiratory insufficiency [1–5]. Nevertheless, complications do frequently occur, and they can lead to intermediate-term or long-term graft dysfunction and decreased survival. According to the International Society for Heart and Lung Transplantation (ISHLT) registry, survival rates at the first, second, and fifth years are 80%, 65%, and 53%, respectively [6]. The prognosis of lung transplant recipients (LTR) has improved considerably in recent years, thanks to the careful selection of donors and recipients, advances in surgical techniques and postoperative care, and better methods for graft preservation.

LT can be either unilateral or bilateral. Single-lung transplantation is generally used for non-septic lung diseases, whereas double-lung transplantation is mandatory for septic lung diseases, such as cystic fibrosis (CF) and bronchiectasis. Infections and episodes of acute rejection are both significant complications soon after LT. Moreover, the main obstacle to the long-term success of LT remains chronic rejection, characterized histologically as bronchiolitis obliterans. It occurs in up to two thirds of patients [7]. The most relevant risk factor for the development of bronchiolitis obliterans syndrome (BOS) after the number of previous acute rejection episodes and the incidence of persistent rejection is cytomegalovirus (CMV) infection and disease [8]. Recent evidence also suggests a possible role for respiratory viruses (RV) as risk factors for chronic rejection in LTR [9, 10]. Finally, a restrictive allograph syndrome came up as a novel phenotype of chronic rejection with worse prognosis than BOS [11].

Infectious complications are a frequent cause of morbidity and mortality and the most prominent cause of death the first year. More than two thirds of them affect the respiratory tract [6, 12].

This chapter focuses on the epidemiology and prevention of bacterial, viral, and fungal infections in lung or lung–heart transplant recipients. Additionally, it addresses specific aspects of donor, residual, or native lung infection or colonization, as well as issues involving recipients with CF. One of the main problems with dealing with infection in LT is the paucity of randomized, controlled studies. So more controlled studies are needed to answer the questions regarding infection in LTR.

11.2 Risk Factors for Infection

The risk of infection in an LTR is determined by interrelationships among numerous factors related to the recipient, associated with the type of transplant and the surgical procedure, and inherent to the infecting microorganism and the state of permanent therapeutic immunosuppression required to avoid graft rejection. Table 11-1 summarizes these risk factor groups.

11.2.1 Recipient-Related Factors

The recipient's pre-transplantation clinical status is important; patients with renal failure, those on mechanical ventilation, and those with morbid obesity or malnutrition have a higher incidence of infection after LT [12–15]. Advanced age is also associated with an increased risk [16]. In some programs, mechanical ventilation is a major contraindication to LT as airway colonization with bacteria may lead to nosocomial infection and the associated respiratory muscle deconditioning may require prolonged postoperative ventilatory support. However, recent results have shown that pre-transplantation mechanical ventilation is not associated with a higher risk of later bacterial infection [17–19]. In fact, nowadays, most programs accept mechanical ventilation as a bridge for LT for candidates previously included on the waiting list.

Various treatments administered to the candidate before LT as well as underlying diseases (such as diabetes mellitus) may be relevant to the type and severity of infection after LT. Candidates treated with corticoids or antimicrobial agents before transplantation have a higher incidence of

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Table 11-1. Risk factors for infection in recipients of a lung or heart–lung transplantation

Recipient
Underlying conditions such as diabetes or hepatitis
Older age
Absence of specific immunity to CMV, HSV, VZV, EBV
Colonization of the recipient by bacteria or fungi
Latent infection due to TB, CMV, VZV, HSV, EBV
Previous therapy with antimicrobial agents, corticoids, or other immunosuppressors
Clinical state of the recipient at the time of transplantation:
Renal failure
Malnutrition
Low vitamin D levels
Obesity
Mechanical ventilation
Transplantation
Preservation lesion
Surgical factors:
Duration of procedure, meticulous technique
Surgical complications: suture dehiscence, hemorrhage, arterial ischemia
Repeated surgery required
Postoperative instrumentation:
Duration of mechanical ventilation
Intravascular catheters
Urethral catheter
Continuous exposure to the external environment
Denervation of allograft:
Diminished cough reflex
Abnormal mucociliary clearance
Reactive hyperresponsiveness
Interrupted lymphatic drainage (especially during first weeks)
Anastomosis site:
May enhance colonization
Airway dehiscence and mediastinitis
Bronchial stenosis and postobstructive infection
Donor lung may transmit infections:
From prolonged mechanical ventilation
From latent infections (TB, CMV, VZV, HSV, EBV)
From previous bacterial or fungal colonization
Native lung after single-lung transplantation:
Occult pretransplant infection (TB, *Aspergillus* spp., *Pneumocystis jirovecii*, etc. especially with immunosuppression before transplantation)
Sinus infection in cystic fibrosis and ciliary dysfunction syndromes
Bronchiolitis obliterans:
Enhanced immunosuppression
Impaired clearance
Bronchiectasis
Immunosuppression
Immunomodulating viruses
Graft rejection

Abbreviations: *CMV* cytomegalovirus, *HSV* herpes simplex virus, *VZV* varicella-zoster virus, *EBV* Epstein–Barr virus, *TB* tuberculosis.

infections due to bacteria and *Candida* spp. [16, 20]. On the other hand, low-dose pre-transplantation corticosteroid treatment has proved to be even beneficial; it allows LT in patients who cannot be completely weaned from such therapy [21]. In cases of single-LT plus pre-transplantation corticosteroid treatment, the remaining native lung may harbor opportunistic microorganisms, including *Aspergillus* spp. (IA), tuberculosis, or *Pneumocystis jirovecii* [22]. Therefore, performing a histopathologic study and culture of the resected lung to rule out these infections and to provide treatment when they are detected is extremely important. Finally, the indiscriminate use of antimicrobial agents before transplantation can lead to the selection of multidrug-resistant (MDR) strains that are difficult to treat. This often occurs in recipients with CF, as discussed below.

The absence of specific immunity in the recipient to some viral infections, especially CMV or Epstein–Barr virus (EBV), implies a higher risk of acquiring these infections when the donor lung harbors latent infection by these viruses. Such primary infection produces disease with greater frequency and more severity than do cases of reactivation.

Vitamin D deficiency is frequent in LT candidates and greater than in general population. Vitamin D plays a role in cell-mediated immunity as well as in innate immune response. A retrospective cohort study showed that 80% of LTR were deficient for vitamin D. Infectious episodes due to bacteria, CMV, fungi, and non-tuberculous mycobacteria (NTM) in this group were more frequent than in the non-deficient group within first year after transplantation (5.41 vs. 3.15; $p > 0.001$) [23].

11.2.2 Transplant-Related Factors

Initial dysfunction of the transplanted organ caused by arterial ischemia or severe preservation lesions secondary to a prolonged interval of ischemia influences the frequency and severity of post-transplant infections. Similarly, alloreactivity reactions against the graft make it more prone to infection by certain viruses. The most frequent sites of infection in the immediate postoperative period are the lung, the pleura, and the extrapulmonary chest cavity, since the integrity of the visceral pleura is not restored and the mediastinal space is lost due to communication with the pleural spaces.

With respect to the interval of graft ischemia, Fiser et al. [24] showed that a cold ischemia time longer than 6 h did not increase the risk of reperfusion injury, acute rejection, CMV infection, bacterial or fungal pneumonia, BOS, 1-month mortality, 1-year mortality, or 5-year mortality, after reviewing data from 136 LTR over a 10-year period. These findings have not been supported by results from other groups [16].

The length and the need for repeated surgery are the most important surgery-related risk factors for the development of

bacterial or invasive fungal infection (IFI) during the immediate post-transplantation period [16].

In LT, several special predisposing factors for the appearance of bacterial pneumonia are present. The state of ischemia for several hours after donor lung extraction, and reimplantation without reestablishment of the graft's lymphatic drainage and innervation clearly affect the graft's defense mechanisms. The airway mucosa is damaged, and the mechanism of mucociliary clearance is paralyzed. Anastomosis of the airway also decreases the clearance of respiratory secretions. Graft denervation eliminates the cough reflex, allowing secretions to accumulate. The interruption of lymphatic drainage prevents the immune system effector cells of the regional lymph system from reaching the lung, which in turn alters the immune response against antigens deposited in the lung [25]. Moreover, the graft's microenvironment consists of human leukocyte antigen incompatibility between the host alveolar macrophages and the donor alveolar lymphocytes [26]. Additionally, small inoculum of microorganisms extracted with the graft can produce severe pneumonia in the already immunosuppressed recipient [27]. Finally, the lung is in constant contact with ubiquitous airborne bacteria.

Finally, the most important predisposing condition for post-transplantation infection is BOS. LTR with BOS are usually profoundly immunosuppressed, and their lung function and mucus clearance are often markedly impaired. In fact, the most common cause of death in patients suffering from BOS is infection.

11.2.3 Lung Transplant Donor

Almost all donor lungs harbor microorganisms at the time of procurement [28]. Thus, the risk of donor-to-host transmission of infection is inherent; this has repercussions on donor selection and on the choice of prophylactic regimens administered to the recipient of a lung or heart–LT.

The authors' group has recorded data from donors of lung allografts transplanted to 49 recipients surviving at least 24 h after LT [28]. Overall incidence of donor infection was 73.4%. The types of donor infection included isolated contamination of preservation fluids (17.9%), graft colonization (69.2%), and bacteremia (12.8%). Donor infection rates did not differ statistically between those mechanically ventilated for 48 h or less or more than 48 h. Donor-to-host transmission of bacterial or fungal infection occurred in 15 (7.6%) LTR (Table 11-2). In our experience, 25% of donors with bacteremia and 14.1% of colonized grafts were responsible for transmitting infection. Two patients died because of transmitted infection (Table 11-2). Microorganisms for which it is extremely difficult to design effective prophylactic regimens caused five cases of infection: *A. fumigatus*, *Stenotrophomonas maltophilia* and methicillin-resistant *Staphylococcus aureus* (MRSA). Excluding these cases, prophylaxis failure occurred in 5.6% of procedures (5.6%).

Similarly, Low et al. [29] reported that 28 of 29 bronchial washings taken from donors grew at least one microorganism. The most common microorganisms identified were *Staphylococcus* spp. and *Enterobacter* spp. In 43% of these cases, similar microorganisms were isolated from the recipient tracheobronchial tree, and, of these, 21% had subsequent invasive pulmonary infections. Waller et al. [30] performed a retrospective comparison of the outcome of 123 donors in 125 consecutive, technically successful lung or heart–LT. Microbial contamination of routine donor bronchial lavage was about 60%. Five recipient deaths were due to donor-transmitted pneumonia.

A bronchial washing or aspiration for microbiologic sampling should be routinely performed in the lung donor to guide the choice of adequate recipient prophylaxis. Gram,

Table 11-2. Description of infection episodes due to donor-to-host transmission

Microorganism	Type of donor infection	Type of recipient infection	Outcome	Prophylaxis
A. fumigatus	Colonization	Tracheobronchitis	Cured	A–A
A. fumigatus	Colonization	Tracheobronchitis	Cured	A–A
A. fumigatus	Colonization	Mediastinitis	Died	A–A
S. viridans	Colonization	Pneumonia	Cured	A–A
MRSA	Colonization	Pneumonia	Died	A–A
S. aureus	Colonization	Pneumonia	Cured	Cefuroxime
S. aureus	Bacteremia	Tracheobronchitis	Cured	A–A
S. aureus	Colonization	Tracheobronchitis	Cured	Cefuroxime
S. aureus	Colonization	Tracheobronchitis	Cured	A–A
S. aureus	Colonization	Cutaneous lesions	Cured	A–A
S. maltophilia	Colonization	Tracheobronchitis	Cured	A–A
P. aeruginosa	Colonization	Tracheobronchitis	Cured	Cefuroxime
P. aeruginosa	Colonization	Tracheobronchitis	Cured	A–A
P. aeruginosa	Colonization	Tracheobronchitis	Cured	A–A
K. pneumoniae + *E. coli*	Bacteremia	Pneumonia	Cured	A–A

Abbreviations: *MRSA* methicillin-resistant *Staphylococcus aureus*, *A-A* amoxicillin-clavulanate + aztreonam.

methenamine silver, calcofluor (for fungi identification), and Ziehl–Neelsen staining; and specific cultures for bacteria, fungi, and mycobacteria should all be conducted [31]. The main problem is that culture results may not be available soon enough. Nevertheless, the finding of positive Gram stain or scanty purulent secretions should not be contraindications for accepting the lung for transplantation [31] because the outcome of these marginally suitable lungs is similar to that obtained with ideal grafts [32]. However, most groups consider the existence of pneumonia, aspiration of gastric juice, abundant purulent secretions that persist after bronchial washing, or the growth of filamentous fungi on a culture of fiber-optic bronchoscopy samples to be contraindications to transplantation. Since two of the authors' patients who received lungs contaminated with *Aspergillus* spp. developed invasive aspergillosis (IA) and died, the group excludes lungs for which calcofluor stain evidences hyphae. The heavy growth of *Candida* species in the donor bronchus makes these lungs risky because of the potential involvement of the vascular sutures or large vessels, which could lead to mycotic aneurysms and consequent rupture [33]; therefore, this represents a significant obstacle for accepting these organs. This is more important for heart–LTR. So, the graft should be excluded if the culture is pure and highly abundant. If this is not the case, an echinocandin should be initiated immediately after transplantation.

An experimental study in canine LT has provided evidence that antibiotic treatment of donors showing bacterial contamination prevents the development of pneumonia in recipients [34]; nevertheless, no consensus has been reached on whether antimicrobial treatment should be used in all human lung donors. Although this measure might decrease the risk of early bacterial pneumonia, it might also induce false negative results in cultures and thus may make recipient management after transplantation more difficult.

11.2.4 Cystic Fibrosis

Chronic infection of the respiratory tract before transplantation distinguishes patients with CF from patients with other diseases. Nonetheless, several studies report that recipients with CF receiving bilateral lung transplants do not have a higher risk of infection after the procedure despite the common presence of airway pathogens (*Pseudomonas* spp., *S. aureus*, and molds). Many patients show the same strains of *P. aeruginosa*, as demonstrated by electrophoretic DNA analysis, after transplantation, probably due to contamination during surgical graft placement or from the chronic sinusitis occurring in these patients [35]. Although the efficacy of surgical sinus drainage has not been established, some recommend this procedure [2–4, 36].

Some centers exclude patients with certain respiratory pathogens, such as *P. aeruginosa* resistant to all antibiotics, or those with other MDR bacteria, including *B. cepacia*, *S. maltophilia*, or *Alcaligenes xylosoxidans*. However, data demonstrating post-transplantation infection and survival rates that are similar to those of patients with sensitive strains suggest that this policy is unwarranted [2, 37–39]. The presence of *B. cepacia* is considered an absolute contraindication to LT in some centers because of its high associated risk of severe and often lethal postoperative pneumonia and sepsis and because transmission between patients is well documented [40]. Recent reports have documented lower survival in recipients previously colonized by *B. cepacia*, and specifically, by *B. cepacia* genomovar III strains [41, 42].

Aspergillus spp. is recovered from respiratory tract cultures in up to 50% of patients with CF. Its presence is not, however, predictive of subsequent allograft infection, and it should not be considered a contraindication to transplantation unless evidence shows mycetomas adhering to the chest wall.

An increase incidence of NTM has been observed [43]. All patients with CF should be evaluated for NTM pulmonary disease before LT. Patients with NTM disease should begin treatment before transplant listing. In case of progressive pulmonary or extrapulmonary disease despite optimal therapy or an inability to tolerate it, LT is a contraindication [44].

11.2.5 Native Lung

In single-LTR, the residual native lung can give rise to a large number of post-transplantation complications. In addition to bacterial or fungal pneumonia and bronchial anastomosis infections leading to dehiscence, the native lung can have noninfectious problems such as severe overinflation, perfusion mismatch, or pneumothorax. The incidence of native lung infectious complications in single-LTR ranges from 20 to 50% [15]. In patients with idiopathic pulmonary fibrosis treated with high-dose steroids, infection of the native lung by *M. tuberculosis*, *P. jirovecii*, and *A. fumigatus* may go unnoticed in the evaluation of the candidate and may result in a serious exacerbation of infection after transplantation. Pathologic and histologic analyses of the resected lung are essential. Native pulmonary aspergillosis is the most feared complication because it is difficult to diagnose and practically impossible to treat unless a pneumonectomy can be performed. Moreover, primary prophylaxis for IA is complex because of the problems in reaching acceptable concentrations of therapeutic drugs and the fact that nebulized amphotericin B (AFB) is not properly distributed in a lung with significant ventilation and perfusion defects.

11.2.6 Immunosuppression

LTR have a permanent deficit of immunity due to the immunosuppressive treatment required indefinitely to avoid rejection.

The use of OKT3 as an induction agent is now very limited due to an increase risk of infection [45]. In contrast, antithymocyte globulin (ATG) and basiliximab do not increase the rate of infections and have been associated with a survival benefit [46].

In patients treated with cyclosporine (CsA) or tacrolimus, the incidence of infection is quite similar [47]. The University of Pittsburgh performed a study that compared the effects of tacrolimus and CsA. The prevalence of bacterial infection was 1.5 episodes per 100 patient days in the CsA group and 0.6 episodes per 100 patient days in the tacrolimus group, although with no statistical significant difference. The prevalence of CMV and fungal infection were also similar in both groups [48].

The role of antimetabolites such as mycophenolate mofetil (MMF) and inhibitors of the mammalian target of rapamycin (mTOR) such as sirolimus or everolimus is discussed below when assessing CMV infection.

The incidence of serum immunoglobulin deficiencies can be as high as 44% in LTR and has been associated with community-acquired respiratory viral infections, IFI and BOS [49, 50]. However, a randomized, double-blind, placebo-controlled trial of immune globulin intravenous administration in LTR with hypogammaglobulinemia failed to demonstrate a reduction in the short-term risk of bacterial infection [51].

Infection by immunomodulating viruses, such as CMV, increases the net state of immunosuppression favoring the development of opportunistic infections [52]. An extensive study performed at the University of Pittsburgh assessed the risk factors for infection other than CMV in 250 transplantations (99 single lung, 102 bilateral lung, and 49 heart–lung) [16]. Early post-transplantation risk factors for infection included CMV mismatch (donor is CMV-positive, recipient is CMV-negative [D^+/R^-]), among others. Risk factors for late infection included again CMV mismatch, the absence of CMV prophylaxis, and CMV disease, among others [16].

11.3 Bacterial Infection

11.3.1 Epidemiology

Bacterial infection is the most frequent infectious complication for lung and heart–LTR. The rate of bacterial infections (mainly respiratory) is much higher than that observed in other SOTR. Of the total infections observed in different series, 35–66% were bacterial, and 50–85% of recipients presented a bacterial complication after transplantation. Frequently, patients experienced more than one bacterial infection and bacterial respiratory infection occurred most frequently [1, 5, 6, 12, 13, 16, 53, 54]. Beginning with persistent colonization, lung and heart–LTR can present with any of the clinical forms of this process (tracheobronchitis, sinusitis, pneumonia).

Table 11-3. Factors related to bacterial infections in LTR

Immediate post-transplant period	Late post-transplant period
Pre-transplantation colonization [55]	Increased immunosuppression due to rejection
The surgical procedure itself and technical complications (e.g., bronchial anastomosis dehiscence) [13]	Invasive diagnostic procedures
Intubation and/or prolonged hospitalization [16]	Development of BOS [16, 56]

Factors related to bacterial infections presenting in the immediate and late post-transplantation are depicted in Table 11-3.

The most frequent causal agents of nosocomial pneumonia are *P. aeruginosa*, Enterobacteriaceae and *S. aureus* [57, 58]. Other prevalent Gram-negative nosocomial bacteria include *Acinetobacter* spp. and *Stenotrophomonas maltophilia*. The period of maximum risk spans the first 3 weeks after transplantation. Nevertheless, its incidence during this interval has markedly decreased with the implementation of antibiotic prophylaxis; most cases of bacterial pneumonia now occur in the intermediate and late postoperative period. In fact, health care-associated pneumonia is more frequent than hospital-acquired pneumonia [58]. Stable, ambulatory transplant recipients after the postoperative phase can develop pneumonia from infection with microorganisms prevalent in the community (e.g., *Mycoplasma pneumoniae*, *Haemophilus influenzae*, or *Streptococcus pneumoniae*). Infection due to MDR bacteria is a widespread problem, especially early after the procedure in the setting of hospital-acquired/ventilator-associated pneumonia (VAP). Its appearance is associated with high rates of morbidity and mortality [59]. These bacteria (e.g., MRSA, MDR *P. aeruginosa* or *B. cepacia*) may colonize the recipient before transplantation (e.g., patients with CF) or can also be acquired after surgery (e.g., MDR *Acinetobacter baumannii*). Our group reviewed VAP incidence, etiology, and outcome in our cohort of LTR. VAP was diagnosed in 20% of LTR. *P. aeruginosa* was the most frequent microorganism isolated (60% MDR), followed by Enterobacteriaceae. Mortality was significantly higher in those patients diagnosed with VAP (OR 9, CI 3.2–25.1, $p<0.01$) [60]. In another study performed in RESITRA (Spanish Research Network for the Study of Infection in Transplantation), evaluating 85 pneumonia episodes in 236 LTR (with an incidence of 72 episodes per 100 patients-per-year), bacterial pneumonia (82.7%) was more common than fungal (14%) or viral (10.4%). Gram-negative bacilli were the etiology in 34 cases (*P. aeruginosa* in 14 and *A. baumannii* in 8). The absence of pneumonia caused by *Legionella pneumophila* was noteworthy and likely due to the effect of cotrimoxazole prophylaxis [61].

The physician must remember that even the growth of normal oral flora in a respiratory sample in the early transplantation period is considered a risk factor for bacterial

pneumonia. Therefore, laboratory workups should identify and perform susceptibility study of all strains isolated. In addition, the clinician should determine whether the anastomosis shows signs of ischemia. If these are present, they imply a greater risk of both infections to the anastomosis and suture dehiscence, and they might be an indication for the use of nebulized antibiotics to treat respiratory colonization or infection.

Deep surgical site infections (SSI) are an uncommon complication in LTR. In a retrospective study at a single center, 5% of LTR developed SSI [62]. Empyema was the most common (42%), followed by wound infection (29%) and mediastinitis (16%). However, the term "thoracitis," rather than mediastinitis, is more accurate because the mediastinal space does not exist as such; during lung implantation, the visceral pleura are not joined to create separate mediastinal space. Therefore, when infection occurs in this extraparenchymal thoracic space, the entire thoracic cavity becomes infected with purulent collections in several locations. Interestingly, 23% of SSI was due to pathogens colonizing recipients' native lungs at the time of transplantation suggesting surgical seeding [62]. One-year mortality associated with SSI was 35% [62].

Bacteremia in LTR is an early complication after transplantation almost related to catheter. The etiology is equally distributed between Gram-negative and Gram-positive bacteria. Nearly half the isolates correspond to MDR microorganisms [63].

Infections caused by *Mycobacterium tuberculosis* are reported because of reactivation, occult disease in the remaining native lung after single-lung transplantation, or transmission by the graft [64]. Within the authors' transplant program, pulmonary tuberculosis is diagnosed in about 6% of LTR. The mean post-transplantation interval at which *M. tuberculosis* is detected is 115 days. In 40% of the cases, the diagnosis was obtained from the explanted lungs. Despite immunosuppression, an adequate response to antituberculous treatment and a low incidence of adverse side effects is observed [65].

Episodic isolation of NTM is common in LTR with an incidence rate of 9/100 person-years [66]. Previous NTM colonization and treated acute rejection are risk factors for NTM disease [67]. The most common NTM isolated is *Mycobacterium avium complex* (69.8%), followed by *Mycobacterium abscessus* (9.4%), and *Mycobacterium gordonae* (7.5%). Most isolates occur among asymptomatic patients and are transient. Nevertheless, NTM disease rate is higher among LTR than in the other SOTR [68]. Moreover, infection due to *Mycobacterium abscessus* is a difficult-to-treat infection. The ISHLT published a study including 5200 LTR. Seventeen patients (0.33%) were identified with *M. abscessus* infection affecting the pulmonary allograft in 12, the skin/soft tissue in 3, or both in 2. Therapies included multiple antibiotics in 16, surgical debridement in 2, interferon-gamma in 1, or no therapy owing to presumed colonization in 1. Ten of 17 patients were considered cured while 2 patients died due to infection [69]. More recently, NTM infection has been associated with increased risk of mortality independent of BOS [67].

Table 11-4. Specific risk factors for *Nocardia* spp. infection in LTR

Frequent episodes of rejection
High-dose corticosteroid treatment
Renal impairment
Prolonged respiratory support

Nocardia spp. infections are uncommon in lung, or heart–LTR. Specific risk factors are shown in Table 11-4. One retrospective review of 540 heart, lung, or heart–LTR examined 10 patients (1.9%) with nocardia infection. It occurred at a median of 13 months after transplantation. All the patients had pulmonary disease and no evidence of extrapulmonary involvement. Nocardia infection did not contribute to patient deaths directly. Coinfection with other pathogens was present in six patients, and two had sequential infections [70]. A chart review from 1990 to 2007 revealed *Nocardia* spp. infections in 4 of 410 LTR despite prophylaxis [71]. All infections were confined to lung and occurred at a median of 315 days after transplantation. *Nocardia nova* was isolated in two patients, *Nocardia farcinica* in one, and unspecified *Nocardia* spp. in one. All isolates were susceptible to cotrimoxazole [71].

The incidence of *Clostridium difficile* infection (CDI) is rising in recent years up to 22.5% in LTR [72] and is higher than in other SOTR with the exception of pancreas [73]. Half the cases presents within the first month after transplantation. Previous administration of cephalosporins and corticosteroid use before transplantation has been considered as risk factors for CDI which, in turn, is not predictive of mortality [72, 73].

11.3.2 Specific Features of Antibiotic Treatment

The forthcoming treatment recommendations, as well as many of the other found in this chapter, are based mainly on the authors' experience in managing these patients and not only on scientific data.

No standardized regimen or guidelines exist regarding the choice of perioperative antibiotic therapy. Antibiotic prophylaxis in LTR should be initiated with broad-spectrum antimicrobials to cover *P. aeruginosa*, and *S. aureus*. For initial prophylaxis, the authors' group uses combined amoxicillin-clavulanate, 2 g, plus aztreonam, 2 g, every 3 h during surgery, and every 8 h thereafter. Recipients with septic lung disease (e.g., CF or bronchiectasis) should receive antimicrobial agents tailored according to their pre-transplantation sputum cultures. In this case, the authors also recommend nebulized tobramycin from the patient's arrival to the ICU

after surgery. The duration of prophylaxis depends on the results of donor and recipient respiratory sample cultures at the time of LT. When cultures are negative, prophylactic agents are withdrawn on the third to fifth days. When cultures are positive or in recipients with septic lung disease, antibiotic treatment is adjusted and maintained for 2 weeks or until cultures are negative. With this approach, the incidence of bacterial pneumonia in the early post-transplantation period (first 3 months) in the authors' lung transplant population is approximately 10%.

Whenever a clinically significant microorganism is isolated in a respiratory sample within the first 3 months, specific intravenous antibiotic therapy is started, even if the patient is asymptomatic. The only situations in which treatment should not be started are colonization with oral streptococci or plasmocoagulase-negative staphylococci. Combined and aminoglycoside treatment should be used for pneumonia. In the case of tracheobronchitis due *P. aeruginosa*, the authors combine a β-lactam with nebulized tobramycin at a dose of 100 mg every 12 h. Other indications in the authors' hospital for nebulized tobramycin or colistin include colonization with MDR Gram-negative bacilli, particularly *Acinetobacter* spp., *Pseudomonas* spp., and *S. maltophilia*; and episodes of tracheobronchitis in which signs of anastomotic ischemia are found.

From the third to sixth month after transplantation, only symptomatic episodes of infection are treated. Colonization is only treated when the microorganism is demonstrated in two respiratory samples taken at 1-week interval.

11.4 Fungal Infections

11.4.1 Epidemiology

Among SOTR, the lung and heart–lung have the highest associated incidence of fungal infection. The etiology is characteristically *Aspergillus* spp., in contrast to others in which infection by *Candida* spp. is the most common. A large study observed a 12-month cumulative incidence of 5.5% of IFI with *Aspergillus* spp. as the leading etiology (72.7%) [74]. Aspergillus infection in LTR is manifested in several ways, including airway colonization and various forms of tracheobronchitis (simple or ulcerative, with or without pseudo-membrane formation). Colonization with *Aspergillus* spp. occurs in 22–85% of LTR at some time after transplantation [36, 75]. Without prophylaxis, the incidence of IA ranges from 13 to 26%, and the related mortality is high (41–100%). With prophylaxis, the incidence of IA is 2–8% [75–77]. The incidence of tracheobronchitis is about 4–12% [75]. In our center, the incidence of IA and tracheobronchitis in 104 LTR given nebulized liposomal amphotericin B (n-LAB) prophylaxis was 0.9% and 1.9%, respectively. IA was classically considered a complication of the immediate post-transplant period, but a RESITRA study demonstrated that its incidence remains high after this period [76]. However, about two thirds of the episodes of ulcerative tracheobronchitis and IA occur at 6–9 months after transplantation. Mortality for tracheobronchitis is around 25%, but for IA rises to 67–82% [78].

Table 11-5. Risk factors for *Aspergillus* spp. infection in LTR

Previous colonization with *Aspergillus* spp. [73]
CMV pneumonitis [22, 78, 79]
Airway ischemia
Single-lung procedure [13, 22]
Single-nucleotide polymorphisms in the genes encoding interleukin-1β and β-defensin-1 [80]
Bronchiolitis obliterans syndrome

Significant risk factors for the development of IA in LTR are listed in Table 11-5. Surprisingly, no relationship with rejection or augmented immunosuppression has been recognized, but this possibility cannot be ruled out. BOS is a risk factor for IA but, on the other hand, LTR colonized with small conidia *Aspergillus* spp. (*A. fumigatus*, *A. nidulans*, and *A. terreus*) are prone to developing BOS [81]. Patients in whom *Aspergillus fumigatus* was isolated from airway samples during the first 6 months were 11 times more likely to develop IA than were those not colonized [79]. The relationship between colonization and invasive disease at 6 months to 1 year after transplantation is not so evident. No difference in the frequency of postoperative colonization is established between recipients with CF and recipients without [75]. The authors' transplant group does not consider previous colonization by *Aspergillus* spp. to be a transplant contraindication; however, in these patients, bilateral lung transplant is mandatory, and chest computed tomography (CT) scanning must be performed to rule out the adherence of mycetomas to the chest wall.

Tracheobronchitis is a characteristic type of aspergillosis almost exclusive to LTR [75, 82]. A spectrum of disease occurs, from simple bronchitis to pseudomembranous, nodular, and finally ulcerative tracheobronchial aspergillosis that is considered a form of IA. The anastomotic site is often affected, and this can lead to suture dehiscence, severe hemorrhage, or disseminated disease, invariably being fatal. Distinguishing between asymptomatic colonization and tracheobronchitis can be difficult as clinical symptoms may be absent or attributed to a concurrent clinical process (e.g., bacterial infection, rejection). When *Aspergillus* spp. is isolated from respiratory samples in the first 6–9 months, the authors perform a bronchoscopic study to rule out pseudomembranous or ulcerative tracheobronchitis. Likewise, early isolation of *Aspergillus* spp. from the airways identifies LTR at increased risk for the development of endobronchial abnormalities such as exuberant granulation tissue or stricture formation [83]. The authors believe that initiating treatment is mandatory whenever this microorganism is isolated from respiratory samples.

The diagnosis of IA is problematic because of the risk of colonization and contamination and the low predictive value of respiratory sample cultures (mainly sputum). For LTR, the presence of a new or progressive infiltrate or consolidation can be taken into consideration for diagnosis, although classical radiological criteria include the appearance of dense, well-circumscribed lesions, cavitations, or endobronchial lesions [84]. Galactomannan (GMN) detection in bronchoalveolar lavage (BAL) is useful in diagnosing IA. The role of GMN quantification was assessed in a study of BAL samples in 116 LTR. The authors found a sensitivity of 60% and a specificity of 95%, based on a cutoff of 0.5, that raised to 98% when the cutoff was 1.0 [85]. Another study reported sensitivity and specificity of GMN in BAL of 100% and almost 91%, respectively, using an index >1.0 as cutoff [86]. Finally, the ISHLT includes pan-*Aspergillus* PCR in BAL together with compatible symptoms and radiological imaging for the diagnosis of probable IFD in LTR based on a study that reported a sensitivity and specificity for diagnosing IA of 100% and 88%, respectively [87]. Nevertheless, the authors consider that PCR techniques warrant further studies and should not be used for routine daily diagnosis or treatment monitoring until standardization is performed.

Another distinctive issue is IA of the native lung in single-LTR. This may develop immediately after transplantation because of preexisting disease that was not detected, or it may represent de novo infection in patients with destroyed native lungs [13, 22, 88]. At times, IA is extremely difficult to diagnose. It occurs in patients with unilateral grafts who are diagnosed with *Aspergillus* tracheobronchitis; because of the unstructured nature of the native lung parenchyma, alterations are difficult to visualize on CT until the process is well advanced. This type of disease has poor prognosis, since achieving therapeutic concentrations of antifungal agents in the residual lung parenchyma is virtually impossible. In cases of tracheobronchitis in single-LTR, the authors employ BAL of each lung and initiate the same treatment used for IA when selective BAL of the native lung culture is positive. It is advisable to perform a native lung pneumonectomy if possible because it probably represents the only way to cure an established process.

Most cases of candidiasis occur during the first months after surgery. The main portal of entry is the gastrointestinal tract, followed by endovascular catheters and the urinary tract. Candida infections can manifest as peritonitis, empyema, candidemia [89], urinary tract infection, necrotizing bronchial anastomotic infection [90], mediastinitis, or esophagitis. Graft-transmitted candidiasis, which ends most often in fungal arteritis, has been described in heart and so can be in heart–lung transplantation [33].

The incidence of *P. jirovecii* pneumonia varies greatly among centers [13]. A prevalence of up to 88% has been described in patients without prophylaxis. Cotrimoxazole prophylaxis is effective in nearly 100% of patients, so its administration is mandatory. About one-third of *P. jirovecii* infections occur after the first postoperative year. Then, since recipients maintain steroid treatment, the authors recommend lifelong prophylaxis [91]. Dapsone may be an alternative for patients with contraindications or intolerability to trimethoprim-sulfamethoxazole.

The incidence of cryptococcosis ranges between 0 and 1.5% in American and European series of SOTR, and it is the third most common infection after candidiasis and IA [92]. The antifungal activity of calcineurin inhibitors may explain this low incidence [93] which, in turn, is higher in heart than in lung transplantation. *Cryptococcus neoformans* var. *grubii* has no particular geographical predilection and causes the most infections. *C. neoformans* var. *neoformans* is prevalent in northwestern Europe. and *C. gattii*, has emerged in the Pacific Northwest [94] and in Europe [95]. Patients who receive high doses of corticosteroids or monoclonal antibodies such as alemtuzumab and infliximab have the highest risk [96]. Cryptococcosis is typically a late-occurring infection; the time to onset usually ranges from 16 to 21 months post-transplantation. More than half of SOTR have disseminated disease or CNS involvement and as many as 33% have fungemia [97]. The mortality of cryptococcosis ranges from 14 to 27% [92, 93].

The incidence of infections by molds other than *Aspergillus* spp. has increased in recent years [98]. Most are caused by Mucorales (mucormycosis or zygomycosis), although infections by *Fusarium* spp. [99] and *Scedosporium* spp. are also recorded. Recent American and European series reported a frequency of mucormycosis lower than 3% among all SOTR with fungal infection [92, 100]. Renal insufficiency, diabetes, and previous administration of voriconazole or caspofungin have been described as independent risk factors for mucormycosis [101]. The most common site of mucormycosis is the lung, with a mortality of 45–50% [101]. Mortality can reach 73% in cerebral forms [102]. Infections by *Scedosporium apiospermum* account for 25% of IFI caused by molds other than *Aspergillus* spp., especially in single LTR and CF [103].

Endemic mycoses can potentially cause infection in LTR. These are especially important in endemic areas of the United States such as the Midwest for histoplasmosis. The first year is the period of highest risk for histoplasmosis as a consequence of reactivation of a latent infection, new exposure or donor-derived infection [104]. Urinary antigen appears to be a better diagnostic tool than the fungal antibody serology in LTR [105]. In patients whose explanted lung is found to have histoplasmosis, antifungal prophylaxis seems effective at preventing reactivation [105]. Coccidioidomycosis is typically acquired when patients are exposed to the desert soil of the Southwestern United States and Northern Mexico. The most common mechanism of infection in LTR is reactivation, but donor-derived transmission has also been reported. Patients, in whom there is evidence of prior coccidioidomycosis, either radiographically or serologically, may require lifelong antifungal prophylaxis [106].

TABLE 11-6. Prophylaxis for *Aspergillus* spp. in the lung and heart–lung transplant recipient [107]

Target population	Antifungal drug	Duration
All recipients	Nebulized liposomal amphotericin B 25 mg[a]	Indefinite or for a minimum of 12 months
Recommended strategy	Three times a week until resolution of bronchial suture	
	Once a week from 2 to 6 months	
	Once every 2 weeks thereafter	
or *Guided prophylaxis* in case of the presence of risk factors		
Induction with aletuzumab or thymoglobulin	Nebulized liposomal amphotericin B 25 mg	Determined by the presence of risk factors
Acute rejection	Three times a week for 2 weeks then once a week	
Single-lung transplant		
Aspergillus spp. colonization		
Acquired hypogammaglobulinemia (IgG <400 mg/dL)		

[a]Considered also nebulized amphotericin B lipid complex 50 mg.

11.4.2 Specific Features of Antifungal Treatment

The first pointto remember regarding fungal infection is that the risk period—a minimum of 1-year post-transplantation—for developing IA is quite long. This fact makes the parenteral administration of antifungal treatment unfeasible. Thus, two alternatives, nebulized AFB and oral voriconazole, remain.

Universal prophylaxis against *Aspergillus* spp. is generally accepted in lung and heart–LTR (Table 11-6) [107]. Since most *Aspergillus* infections in LTR affect the respiratory tree and airway colonization by the conidia precedes the infection, nebulized AFB appears to be an attractive approach. The authors' group conducted a study to evaluate the pharmacokinetics and distribution of nebulized AFB in LTR [108]. Airway concentrations of AFB after nebulization with 6 mg of AFB deoxycholate theoretically offer adequate protection. Concentrations in the alveolar lining were higher than those found in the proximal bronchial tree, but the latter were still sufficient to protect anastomoses. Additionally, distribution studies using ventilation and perfusion gammagraphy imaging with technetium-99 m-labeled AFB deoxycholate were performed. All demonstrated acceptable delivery of the agent to native lungs and allografts in amounts proportionate to their degree of ventilation. Prophylaxis with nebulized AFB decreased the incidence of IA below 3% [108]. In the authors' experience, the incidence of any kind of *Aspergillus* spp. infection in 226 consecutive LTR was 7.5%. However, administration of nebulized AFB every 8 h day after day is a considerable drawback. With the aim of prolonging the dosing interval, our group determined the airway concentrations of the drug after nebulization of 24 mg of the liposomal formulation (Ambisome®) [109]. We could demonstrate that AFB concentrations after n-LAB remained high enough for prophylaxis of *Aspergillus* spp. infection over 14 days. There was no significant systemic absorption of the drug and no effect was observed on respiratory function. Thus, the main advantages of nebulized prophylaxis are the lack of drug–drug interactions, the cost-effectiveness relationship, and the ability to achieve high levels of lung antifungal concentrations without systemic side effects [109]. One disadvantage is local irritation that leads to cough or bronchospasm. These effects occur in fewer than 10% of patients. The use of salbutamol or halving the drug concentration can improve the symptoms. Other disadvantages are the need for appropriate equipment and for the patient or family members to know how to administer it. The possibility of irregular distribution of the drug in the lung is another potential limitation [110]. Voriconazole is an alternative although there is also a lack of randomized studies [111]. Moreover, an increase in liver enzymes has been observed in up to 60% of LTR receiving voriconazole leading to discontinuation of the drug in 14% of them [112]. Skin cancer has also been reported in LTR with its prolonged use [113].

Colonization with *Aspergillus* spp. must be treated to prevent IA. The authors recommend n-LAB 25 mg/24 h for 7 days, then 25 mg/72 h, or nebulized AFB lipid complex 50 mg/24 h once every 2 days. In the case of intolerance, voriconazole should be considered (loading dose 400 mg/12 h PO, then 200 mg/12 h PO).

In the case of nodular or ulcerative tracheobronchitis, voriconazole plus nebulized lipid formulations at the doses above mentioned are recommended. A bronchoscopy should be performed every week or every 2 weeks to evaluate the extension of disease and to clear necrotic debris and fungus balls. A high-resolution CT scan should also be performed to rule out parenchymal extension.

In LTR with anastomotic tracheobronchitis due to *Candida* spp. the recommended treatment is n-LAB 25 mg three times a week, or nebulized AFB lipid complex every other day plus removal of the debris by repeated bronchoscopies. Echinocandins may be more effective than azoles for *Candida* spp. growing in the biofilms of the anastomoses.

When dehiscence of the bronchial anastomosis occurs, both surgical resection and stent placement may be necessary in addition to antifungal therapy, although the prognosis is poor. Other indications of surgery are shown in Table 11-7.

Table 11-7. Indications for surgery in IA

Massive hemoptysis
Hemoptysis due to lesions located near large vessels
Isolated or cavitated pulmonary lesions that progress despite the administration of appropriate antifungal treatment
Sinus disease
Infiltration of the pericardium, large vessels, bone, or subcutaneous thoracic tissue while receiving treatment

Table 11-8. Risk factors related to CMV replication and disease in LTR

CMV serology mismatch: positive donor and negative recipient
Absence of specific cell-mediated immunity [115, 116]
Cytolytic agents such as OKT3 [117] or antithymocyte globulin [118]
Acute rejection and its subsequent treatment with corticosteroids
MMF when the dose exceeds 2 g/day
mTOR inhibitors is associated with a lower risk

11.5 Viral Infections

11.5.1 Epidemiology

The second most frequent cause of infection after LT is CMV. The overall incidence of replication and disease without prophylaxis ranges from 53 to 75% [114], a much higher rate than those associated with other SOTR with the exception of small bowel transplantation. In patients without prophylaxis, the incidence of pneumonitis approaches 100% in CMV D^+/R^- cases, but, in contrast to other types of transplant, CMV-positive recipients also have a high incidence, estimated at 60–75%.

The risk factors for CMV disease (Table 11-8) have not been extensively studied in LT, but knowledge obtained in other SOTR can be applied. The most important risk factor for the development of CMV disease is CMV mismatch, which confers more than 50% risk in the absence of antiviral prophylaxis or preemptive treatment strategies [119]. However, cell-mediated immunity is known to be more important than humoral immunity in controlling CMV. CMV infection elicits a strong virus-specific $CD4^+$ and $CD8^+$ T-cell response that, currently, can be measured [120, 121]. As an example, those LTR considered negative or indeterminate to Quantiferon-CMV are at risk of developing CMV disease [115, 116]. In a trial comparing sirolimus to azathioprine, the overall incidence of any CMV event was lower in the sirolimus arm at 1 year (RR = 0.67, CI 0.55–0.82, $p > 0.01$) [122]. The relationship between CMV disease and other risk factors such as co-infection with Human Herpesvirus 6 (HHV-6) [123], hypogammaglobulinemia [124], polymorphisms in toll-like receptors (TLR2 and TLR4) [125], or low levels of mannose-binding lectin [126] has been demonstrated in other types of SOTR rather than lung. Thus, HHV-6 was not detected in 145 samples from 26 LTR, even though 30% of the samples were from 9 CMV DNA-positive patients in whom 13 episodes of CMV pneumonitis were recorded [127].

Transplantation of organs containing a large number of certain cells that can harbor latent or replicating CMV (e.g., macrophages and lymphoid cells) may provide the recipient with a higher initial CMV viral load, which then undergoes reactivation. Similarly, recipients with active CMV infection at the time of transplantation have a higher risk of post-transplantation CMV disease [128]. CMV viral load is an important and clinically useful correlate of CMV pneumonitis in LTR [129–131].

CMV pneumonitis is the second leading cause of pneumonia [15] and the most frequent disease in LTR without prophylaxis. The use of prolonged valganciclovir prophylaxis has changed the incidence of pneumonitis that has decreased in contrast with the viral syndrome that has increased. In addition, the time at which the disease appears is from 2 to 4 weeks after stopping prophylaxis. In the authors' experience, approximately 10% of episodes has a late onset, appearing during the second year. Encountering CMV disease after the second year is exceptional. CMV pneumonitis has an insidious onset, which is manifested by constitutional symptoms and fever, with a later progression to dyspnea and tachypnea. The only relevant sign in an otherwise normal respiratory auscultation is tachypnea. Arterial hypoxemia is almost always present. The clinician should remember that, when a sudden deterioration of respiratory function is observed during treatment for CMV pneumonitis, superinfection by Gram-negative bacilli or fungi must be investigated. The radiologic manifestations of CMV pneumonia are diverse. Bilateral, symmetric, interstitial, and/or alveolar infiltrates predominating in both lung bases are the most common radiologic features.

The diagnosis of CMV disease is based on the definitions that were established by Ljungman et al. [132]. Several studies have shown that quantification of the CMV load in the plasma or blood can be helpful in making the diagnosis and that it can even be used to anticipate the development of CMV disease [129–131, 133].

Ganciclovir-resistant CMV infection, an emerging problem in the transplantation setting, has been associated with CMV D^+/R^- status, a high CMV load, and prolonged exposure to ganciclovir. Limaye et al. described a nearly 10% rate of ganciclovir-resistant CMV infection, as defined by a UL97 mutation, and this was more frequent among D^+/R^- patients despite preemptive antiviral therapy or prophylaxis. Compared with other SOTR, ganciclovir-resistant CMV in LTR include an earlier onset (median of 4.4 vs. 10 months) and less-prolonged exposure to ganciclovir (median of 100 vs. 194 days) [134]. A trend toward more frequent detection of MDR and co-circulation of multiple resistant strains has been also shown in LTR [135].

CMV infection has also an indirect effect on the patient's immune state. The immunomodulation exerted by CMV has

two demonstrated effects. CMV infection induces a transient state of additional immunosuppression that makes the host more susceptible to the development of infection by opportunistic microorganisms [136], and it seems to play a role in the pathogenesis of graft rejection [114, 137]. The detection of CMV DNA in the BAL is associated with the development of BOS irrespective of the magnitude of viral replication, the presence of tissue invasive disease or whether viral replication is symptomatic or asymptomatic [138]. The association between augmented antiviral prophylaxis and reduced cellular rejection has been also identified in LTR [139, 140]. Although antiviral drugs adequately suppress CMV replication, LTR remain vulnerable to both clinical and subclinical CMV replication on cessation of prophylaxis that is associated with BOS. However, other studies [141] reported no increased risk of BOS in a cohort of patients with beta herpesvirus (CMV, HHV-6, and HHV-7) replication within the lung allograft.

Respiratory viruses have been increasingly recognized as common pathogens in LT. Previous cohorts have reported an incidence of RV infections in LT in the range of 7.7–64% [142, 143]. Our group conducted a 5-year prospective study including 98 LTR that demonstrated an overall incidence of RV of 0.85 per patient-year. Our results are similar to data recently published in another large prospective study [144]. Seasonal patterns of RV circulating in LT are comparable to those observed in the community. Picornaviruses (mainly rhinovirus), coronaviruses, and influenza virus were the most common etiological agents, accounting for 76.5% of microbiologically confirmed symptomatic infections. Rhinoviruses are the leading cause of RV infections. Rhinovirus were associated not only with mild self-limiting upper respiratory tract infection but also with lower respiratory tract infection, mainly in form of tracheobronchitis. Patients with paramyxovirus (especially respiratory syncytial virus) and influenza infection had a higher incidence of pneumonia and hospitalization rate [145–147]. The relationship between RV infections and acute rejection has not been clearly established in previous studies [144, 148, 149]. Our data showed a trend toward a significant clinical link between RV and biopsy-proven acute lung rejection including the acute phase of the viral infection (with no relation to clinical presentation) and a follow-up period of 3 months. It has also been advocated that patients with documented community respiratory viral pneumonitis are predisposed to high-grade BOS development [150]. Finally, RV infections have been described as a risk factor for developing bacterial and fungal superinfection [151].

The incidence of pneumonitis due to HSV type 1 ranges from 5 to 10% in LTR without prophylaxis [12]. Most of them are reactivations that appear in the first 2 months, but they can occur as early as 5–10 days after transplantation. HSV pneumonitis is often associated with bacterial or CMV pneumonia. In contrast to CMV pneumonitis, pulmonary involvement by HSV type 1 provokes respiratory insufficiency and bilateral alveolar infiltrates in the majority of patients affected. Valganciclovir prophylaxis for CMV disease also protects against HSV infection.

The incidence of EBV-related post-transplant lymphoproliferative disorders (PTLD) varies greatly, ranging from 2 to 33% [152, 153]. These differences are probably due to variations in immunosuppression regimens, the number of EBV seronegative recipients, and the percentage of pediatric patients included in the series. The risk for developing PTLD is higher in EBV seronegative LTR. However, late onset PTLD tends to present in seropositive recipients. Early onset PTLD involves predominantly the transplanted lung, whereas late onset PTLD does not [154]. Possible enhancement of EBV activity by the β-herpesviruses, such as CMV, HHV-6, or HHV-7, has not been conclusively established. Monitoring EBV DNAemia does not predict PTLD [153].

The incidence of pneumonitis due to adenovirus is quite low, affecting about 1% of all adult LTR. It tends to appear in the first 3 months after transplantation. It induces severe disease with progressive respiratory failure; in most cases, the clinical course is fatal. In contrast, adenovirus infection is a widespread problem in the pediatric LTR. The attack rate is almost 50%, and at least half of the patients die of respiratory failure because of diffuse alveolar damage. BOS develops uniformly in the survivors.

Recently, the experiences of 239 LTR with herpes zoster infection have been published. The calculated incidence was 55.1 cases per 1000 person-years of follow-up. The cumulative probability of herpes zoster was 5.8% at 1 year, 18.1% at 3 years, and 20.2% at 5 years' post-transplantation. Only 5.7% of the patients had disseminated cutaneous infection and none had visceral involvement. Recurrence of herpes zoster was observed in 13.8% of patients. Postherpetic neuralgia was detected in 20% of cases [155].

11.5.2 Specific Features of Antiviral Treatment

Two strategies exist for the prevention of CMV disease in SOTR. The first is prophylaxis, in which an antiviral agent is administered immediately after transplantation to those recipients at high risk for CMV disease (e.g., D^+/R^- cases or patients who require the administration of conventional T-cell receptor antibodies). The second strategy, preemptive therapy, consists of the administration of an antiviral agent when nucleic acid testing (NAT) evidences a level of viral replication highly predictive of CMV disease. An international survey showed the lack of uniformity when managing with CMV infection in LT. Although prophylaxis is the most commonly used preventive strategy, its duration is extremely variable (from 3 months to indefinite). Half the centers routinely decreased immunosuppression at the time of viremia while the other half did not take any measure [156]. In an attempt to avoid this issue, guidelines have been published [157].

The authors believe that prophylaxis plus preemptive therapy is the best strategy for the prevention of CMV disease in LTR. The authors' recommendation is intravenous ganciclovir at a dose of 5 mg/kg every 24 h until oral intake is tolerated, followed by a switch to valganciclovir at a dose of 900 mg once a day until 180 days after transplantation for seropositive recipients and 360 days for CMV mismatch. Valganciclovir at reduced doses to avoid toxicity should not be administered due to its association with CMV disease and increased risk of emergence of resistance [158]. During prophylaxis, we do recommend monitoring of viral load by NAT due to the possibility of breakthrough disease, particularly in seronegative recipients [158]. Once prophylaxis ends, surveillance should continue at every medical visit until the second year after transplantation and preemptive therapy with valganciclovir should be initiated. Treatment is initiated in the following situations: (1) in D^+/R^- transplant recipients, whenever evidence of viral replication is found, and (2) in CMV-seropositive recipients, when viral load is high (e.g., >5000 UI/mL in plasma) or when an increase is registered in two consecutive analyses. When the viral load is under the established cutoff for initiation of preemptive therapy, the analyses should be repeated within 1 week. The duration of preemptive therapy has not been established, but the authors prefer a minimum of 7–10 days when viral replication is negative.

When CMV disease is diagnosed, treatment is started with ganciclovir at 5 mg/kg every 12 h. Generally, tacrolimus and prednisone doses are not reduced, except in cases of pneumonitis, in which they are progressively tapered. MMF is withdrawn or the dose is halved. If ganciclovir-associated leukopenia develops and the polymorphonuclear count drops below 500 cells/mL, the patient is treated with granulocyte-stimulating factor until the polymorphonuclear count increases to more than 1000 cells/mL. In patients with pneumonitis, gammaglobulins at a dose of 200 mg/kg every 48 h are added during the first week of treatment. The viral load should be monitored, and a significant increase after the fourth or fifth day of treatment should raise the suspicion of ganciclovir-resistant CMV infection. However, an increase in viral load during the first 2 or 3 days of treatment is not infrequent. The duration of therapy is usually 15 days, except in cases of pneumonitis, in which therapy is prolonged to 21 days. Generally, viral replication is negative at the end of treatment.

Current evidence, although not based on high-quality studies, suggests that some benefit is derived from the use of oral ribavirin in LTR with non-influenza RV infections [159], especially respiratory syncytial virus [160].

11.6 Conclusion

Despite several advances in surgical technique, immunosuppression and prophylaxis, infection continues to be an important cause of disease and death in LTR. Avoidance of these infectious complications may not only lead to a decrease in the direct consequences of infection but also to a reduction in the subsequent causes of ultimate graft failure including both acute and chronic rejection. There is a need to explore new fields such as the relationship between microbiome and BOS, or to find new and better antivirals, especially for RV infections. But, without forgetting that there are current concerns that must be addressed such as the growing problem of antimicrobial resistance for which careful antibiotic stewardship is mandatory.

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12
Infections in Kidney Transplant Recipients

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Kidney transplantation is the most common type of transplant performed worldwide. Since 1988, more than 370,000 kidney transplants have been performed in the United States [1]. Kidney transplantation has been shown not only to benefit a patient's quality of life but is also more cost effective than dialysis. Transplantation can be performed using deceased or living donor kidneys. The native kidneys are generally left in situ and the transplant kidney is grafted in the right lower abdominal quadrant. The transplant procedure generally consists of anastomoses of the renal artery and vein to the native external iliac artery and vein, respectively. The donor ureter is anastomosed to the recipient bladder.

Posttransplant infections in renal transplant recipients occur in 44.9–81 % patients and include urinary tract infections (UTIs), bacteremia, pneumonia, wound infection, and cytomegalovirus (CMV) infection [2–6]. Severe sepsis posttransplant often causes graft dysfunction [7]. Although there is no specific classification of infections post kidney transplant, these generally follow the timeline of infections post solid organ transplant described previously by Fishman and Rubin [6, 8], with some caveats specific to renal transplantation. The specific infections unique in some aspect to kidney transplantation will be the focus of this chapter. Other infections that are common to all transplant patients are discussed briefly and serve to provide a contextual basis for understanding the global infectious disease burden in kidney transplant recipients.

Postoperative complications and early UTIs are seen in the first month posttransplant. Donor-derived infection should also be considered early in the posttransplant period. During months 1–6, opportunistic infections such as reactivation of herpesviruses, BK virus, and fungal and mycobacterial infections are seen. However, it is important to note that with ongoing prophylaxis and the use of potent antirejection therapies, the initial onset of some infections such as >*Pneumocystis jirovecii* and cytomegalovirus reactivation can occur after 6 months.

12.1 Pretransplant Evaluation of the Kidney Recipient

The pretransplant evaluation of the kidney transplant patient includes obtaining a history of infectious diseases, infectious exposures, and immunizations [9]. Generally, active infectious diseases should be resolved and/or adequately treated prior to undergoing kidney transplant. During the evaluation for transplant, serologic screening for human immunodeficiency virus (HIV), hepatitis C virus (HCV), hepatitis B virus (HBV), human T-cell lymphotropic virus (HTLV), CMV, EBV, herpes simplex virus (HSV), Varicella-Zoster virus (VZV), and syphilis is done and each result needs to be carefully evaluated. HIV is no longer a contraindication to kidney transplant and is discussed further in section Kidney Transplantation in the HIV-Positive Recipient [10]. The knowledge of CMV and Epstein–Barr virus (EBV) serologic status is important to guide antiviral prophylaxis posttransplant. Hepatitis C antibody and hepatitis B surface antigen positivity are not contraindications to renal transplant but the extent of liver disease should generally be delineated with pretransplant liver biopsy. If possible, attempts to treat these viruses should be made prior to transplant. Studies indicate that treatment of HCV with interferon-α and ribavirin posttransplant leads to a 60–70 % rate of allograft rejection [11, 12]. However, this is not an issue with the new protease inhibitors for HCV [13]. On the other hand, hepatitis C-positive recipients could be considered for a kidney transplant from a hepatitis C-positive donor.

Persons who are HTLV-I or -II positive should be assessed on an individual basis. In endemic areas, there is a risk of progression of 2–4 % to HTLV-I-associated myelopathy/tropical spastic paraparesis [14]. A positive syphilis screening test should lead to a confirmatory test specific for syphilis antigens. If a confirmatory test is positive, the patient should be treated prior to transplant. Tuberculosis (TB) skin testing should be routinely performed although a positive skin test is not a contraindication to transplant. False negative skin tests

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can occur in hemodialysis patients [15–18]. Interferon-γ release assays (IGRAs) for TB may also be used for screening in this population. These include the QuantiFERON-TB Gold and T.Spot.TB, both of which measure the amount of interferon-γ released in response to ex vivo cell stimulation with TB-specific antigens. Recent studies have shown that these assays may have improved test characteristics in the hemodialysis population when compared to the tuberculin skin test (TST) [15, 16]. Patients found to have latent TB can be initiated on therapy prior to transplant and complete the course posttransplant if necessary. This consists of isoniazid 5 mg/kg once daily or 900 mg thrice a week (plus vitamin B6) for 9 months [18]. A shorter 4-month course of rifampin may also be effective; however, rifampin will have significant drug interactions with immunosuppressives if transplant occurs while on treatment. An immunization record should also be obtained to ensure routine vaccinations are up to date [19]. Pretransplant, patients should have received tetanus toxoid and pneumococcal vaccine. Immunity to varicella, measles, mumps, and rubella should be determined. If immunity is absent, then varicella and MMR vaccines should be given; however, since these are live vaccines, the transplant should be on hold for 4 weeks after vaccine is given. Hepatitis A and hepatitis B vaccines should also be updated prior to transplant.

12.2 Donor Screening and Donor-Derived Infections

There are some important considerations with regard to donor infections and transmission in the context of kidney transplantation, which may be unique compared to other organs. First, since an alternative exists to kidney transplantation, that is, dialysis, the willingness of physicians or patients to undertake potential risks of infectious diseases transmission associated with certain types of donors may be different than those for other organs. For example, the risk benefit consideration for a critically ill patient with heart failure may be very different than a patient on dialysis. Second, since for deceased donors, usually two kidneys are transplanted, the opportunity exists for early diagnosis of a donor-derived infection that may be transmitted through the allograft, since both recipients may become ill at similar times.

Deceased kidney donors require appropriate screening for infectious diseases [9]. A history should elicit cause of death as well as previous infectious exposures including those potentially acquired during previous travel such as malaria, TB, and rabies. Donor screening generally includes serologic studies for HIV, HCV, HBV (surface antigen and total core antibody), CMV, EBV, and syphilis (discussed in detail in Chap. 7). Additional screening may include West Nile virus (WNV) nucleic acid testing (NAT), which may be dependent on local WNV activity, and the particular policies of the organ procurement organization. HTLV I and II screening is also done in some jurisdictions. Deceased donors should also undergo screening blood and urine cultures. Donors with a history of high-risk behavior may undergo additional testing (NAT) to determine whether they are in the "window period" of seroconversion for HIV, HBV, or HCV. For some OPOs it may be routine to offer NAT testing for all donors. Controversy exists whether organs from increased risk donors should be used for kidney transplantation or not. However, based on a decision analysis, utilization of high risk organs is beneficial even in kidney transplant recipients [20]. A standardized informed consent may increase patient acceptability of these organs [21]. Organs from HCV-positive donors may be considered for use in HCV-positive kidney transplant recipients. Alternatively, the situation may arise where a donor may have previously been treated for HCV and achieved sustained virologic response. In this case, it is controversial whether the kidney should be transplanted in a HCV-negative recipient, since the risk of transmission is largely unknown [22]. Recent consensus guidelines indicate that individual consideration should be given to use of isolated hepatitis B core antibody-positive donors with antiviral prophylaxis in the recipient as the risk of active hepatitis B is low [23]. It is impractical to screen donors for TB using TST; deceased donor screening with Quantiferon-TB in the research setting results in a high number of indeterminate tests [24]. Bacteremic donors are generally acceptable with antibiotic treatment of the recipient [25]. However, caution should be used when donors are bacteremic with multidrug-resistant organisms.

Unusual pathogens such as rabies and lymphocytic choriomeningitis virus (LCMV)/arenavirus have been transmitted to renal transplant recipients, although these cases are rare and difficult to predict [26–31]. There have been no LCMV seroprevalence studies in donors and it is unknown whether donors with rodents are at greater risk. To avoid transmission of unusual viruses, we recommend not to use organs from donors who had died of an unknown form of meningitis or encephalitis. Other pathogens that have been transmitted via donors to kidney transplant recipients include malaria and syphilis [32–35]. These are generally treated successfully if they are recognized early. Unusual fevers or illnesses posttransplant, especially in the first month posttransplant, are alerts for donor-derived infections. In these cases, it is important to revisit the original donor evaluation as well as to investigate whether recipients of other organs from the same donor are experiencing similar illness.

Living kidney donors also undergo screening similar to that of cadaveric donors. However, living donors should also be screened for latent TB using a TST. If determined to have latent TB infection, the donor should ideally complete therapy for latent TB prior to donation [36]. As an alternative, the recipient can be treated with isoniazid for 9 months posttransplant. In the latter case, treatment should be initiated as soon as possible posttransplant since the greatest risk of TB

reactivation is in the first year [37]. *Strongyloides* sp. antibody testing and screening for *Trypanosoma cruzi* (the agent of Chagas disease) should also be done in living donors from endemic areas [38].

12.3 Technical Complications Leading to Infection

Technical problems after kidney transplantation can arise due to either vascular or nonvascular complications. Infections related to these complications usually, but not always, present in the early postoperative period. Overall, the risk of such complications is generally lower than that of other types of transplants. Surgical wound complications are probably the most common and include wound infection, dehiscence, incisional hernias, and lymphoceles [39, 40]. Ureteral complications include urinary leaks and ureteral strictures. Other postoperative issues include vascular thrombosis or bleeding and hematoma formation. In one retrospective study of 870 patients who underwent deceased donor kidney transplant, at least one surgical complication occurred in 34 % [40]. Wound complications occurred in 10.5 % with isolated lymphoceles in 6 %. Risk factors for wound complications include obesity, older age of donor and recipients, as well as certain immunosuppressive drugs such as mycophenolate mofetil (MMF) and sirolimus [40, 41]. The incidence of posttransplant lymphocele is 0.6–18 %, the majority of which are small and asymptomatic [42]. However, approximately 6 % can be infected [43]. Generally, asymptomatic lymphoceles can be followed by ultrasound although clinical symptoms or unresolving lymphoceles should lead to further investigation with percutaneous aspiration and culture. Urinary leaks usually occur at the anastomosis (at the site of vesicoureteral junction) and may occur due to ischemia of the ureter, and can lead to the formation of a urinoma. Although uncommon, urinomas occasionally become infected primarily with Enterobacteriaceae, although other organisms may be seen [44]. Strictures also occur primarily at the anastomosis of the ureter to the bladder, and may be secondary to ischemia or rarely due to BK virus, and lead to recurrent graft pyelonephritis.

12.4 Urinary Tract Infections

By far, the most common infection in a renal transplant recipient occurs in the urinary tract. Incidence has been estimated to be 4–86 % in some series [45–47]. Risk factors for UTI can be divided into pretransplant, intraoperative, and posttransplant factors. Pretransplant factors include female sex, diabetes mellitus, pretransplant immunosuppression, urinary tract abnormality, and dialysis [45, 48]. Intraoperative factors include use of a JJ stent, prolonged catheterization, infected organ, and retransplantation. Routine ureteric stenting during transplant has been shown to decrease the risk of urologic complications but results in a 1.5 times increase in relative risk of UTI [49]. Postoperatively, the risk of UTI is increased if graft dysfunction is present. UTIs can occur at any time posttransplant and timing may in part be dependent on the use of prophylaxis. Symptomatic patients with cystitis may have dysuria, hematuria, frequency, urgency, suprapubic pain, or foul urinary odor. A urinalysis generally shows pyuria and a urine culture reveals significant bacterial growth. Significant growth in the nontransplant literature is generally defined as $>10^5$ colony forming units/mL of urine (or $>10^8$ cfu/L) of a single organism. Significant pyuria is defined as >10 WBCs per hpf. However, lower bacterial colony counts, and limited detection of pyuria, may also occur in renal transplant recipients with significant infection. Acute allograft pyelonephritis is diagnosed if in addition to the abovementioned clinical picture, fever or tenderness over the allograft is present. Bacteremia and a decline in renal function may also be the features of acute pyelonephritis. Atypical presentations are common, and include isolated febrile syndromes, isolated graft tenderness, and other presentations. Emphysematous pyelonephritis is a rare entity that can occur in kidney transplant recipients and often requires transplant nephrectomy in addition to antimicrobials [50, 51]. However, conservative management with antimicrobials and percutaneous nephrostomy has also been reported [52].

Antimicrobial therapy for simple UTIs or graft pyelonephritis should be directed at the organism recovered in urine culture. The duration of therapy for UTI in renal transplant recipients has not been well studied. Graft pyelonephritis can usually be treated with a 2- to 3-week course of appropriate antibiotic therapy. However, longer durations of antimicrobial therapy can generally be used for severe allograft pyelonephritis, recurrent UTIs, and those with structural abnormalities, such as ureteric stents, ureteric strictures, and stones.

Several studies have examined the consequences of UTI in the kidney transplant population. In the first 6 months, UTI appears to be associated with bacteremia and acute rejection [53]; UTI occurring after 6 months (termed late-onset UTI) is shown to be associated with death and graft loss in a large retrospective study [54]. Several mechanisms are postulated for impaired graft function including production of inflammatory cytokines and free radicals or associated acute rejection [45, 55]. Studies have shown that acute graft pyelonephritis can have a deleterious effect on long-term allograft function [56–59]. Giral et al. [57] reviewed 1387 renal transplant recipients, of which 13 % developed allograft pyelonephritis during the follow-up. Pyelonephritis within the first 3 months was significantly associated with poorer graft outcome. Microbial virulence factors have also been associated with acute allograft injury. In one study characterizing *Escherichia coli* isolates from kidney transplant recipients with UTI, the expression of P fimbriae in these isolates was significantly associated with acute allograft injury [60].

To prevent early UTIs, antibacterial prophylaxis with trimethoprim-sulfamethoxazole (TMP/SMX) up to 1 year posttransplant has been advocated by some investigators [61–63]. A small randomized trial of low-dose versus high-dose TMP/SMX showed a significant decrease in UTI occurrence in the high-dose TMP/SMX group (49.2 % versus 25 % patients, $P<0.05$). This suggests that doses used for *Pneumocystis* sp. prophylaxis do not necessarily prevent UTI. This approach is also limited by rising antimicrobial resistance to TMP/SMX. In one single-center review of UTIs in 161 kidney transplant recipients, 25 % of patients developed UTI despite receiving TMP/SMX prophylaxis [64]. Ciprofloxacin is also shown to be effective although quinolone prophylaxis would not prevent pneumocystis infections [65]. In a retrospective review comparing kidney transplant patients that received TMP/SMX versus ciprofloxacin prophylaxis, the latter group was found to have significantly less UTIs at 1 year posttransplant [66]. However, rising rates of fluoroquinolone resistance in solid organ transplant recipients were found in a study of gram-negative bacteremia and may also limit their use [4]. The increasing prevalence of multidrug-resistant organisms including carbapenem-resistant enterobacteriaceae is a major concern in many centers [67, 68]. In small case series, fosfomycin has been used safely to treat drug-resistant infections [69].

12.5 Asymptomatic Bacteriuria

Many kidney transplant programs will do routine urinalysis and culture frequently in the initial postoperative period and continue to monitor at regular intervals thereafter. There is no clear consensus on whether to monitor and if done then how often to monitor the UTI [70]. In many cases, a urine culture may be obtained routinely at the time bloodwork is drawn regardless of patient symptoms. It is unclear how many cases of asymptomatic bacteriuria progress to symptomatic infections or allograft pyelonephritis. However, many physicians will err on the side of treatment especially in the early posttransplant period [48]. Although there is little evidence to support this approach, early in the postoperative period, multiple factors may be present, such as induction immunosuppression, indwelling urinary catheters, urinary stents, and delayed graft function. One small study found that asymptomatic bacteriuria early posttransplant may be a risk factor for symptomatic UTIs—although not with the same organism [71]; others have found no benefit of treating asymptomatic bacteriuria [72]. Ultimately, although there is no clear consensus whether asymptomatic bacteriuria should be treated, there is evidence to suggest that subclinical UTIs may cause allograft damage. One study has shown increased levels of urinary inflammatory cytokines in patients with asymptomatic bacteriuria versus controls [55]. Dupont et al. [73] showed that allograft scarring could occur with asymptomatic bacteriuria even in the absence of vesicoureteral reflux and suggested prophylaxis for asymptomatic infection. Another situation where asymptomatic bacteriuria should be treated is in pregnant transplant recipients.

12.6 Recurrent Urinary Tract Infections

Recurrent UTIs in a renal transplant recipient is defined as ≥3 UTIs per year. Predisposing factors that should be ruled out include vesicoureteral reflux, neurogenic bladder, structural abnormality such as the presence of a ureteric stricture or calculi, or chronic bacterial prostatitis. A persistent renal or perirenal abscess could also serve as a focus of infection for recurrences. Patients with underlying polycystic kidney disease may have cyst infection of the native kidney. Ideally, abdominal imaging with a CT scan or ultrasound should be done to evaluate the transplanted and native kidneys. Referral to a urologist for cystoscopy may be necessary to rule out other structural abnormalities. In many cases, no correctable abnormality is found. If relapse occurs after a 2-week course of antibiotics, then a 4- to 6-week course of antibiotics can be attempted. In a few cases, patients will require long-term antibiotic prophylaxis. The goal is to suppress bacteriuria and one potential approach is to obtain routine cultures while on prophylaxis to see if bacteriuria is suppressed. If prophylaxis is instituted, patients should be reevaluated at regular intervals (e.g., every 6 months) to determine the need for ongoing therapy. If bacteriuria persists or a relapse occurs while on prophylaxis, then this strategy needs to be re-evaluated. Prophylaxis options include amoxicillin, fluoroquinolones, oral cephalosporins, TMP/SMX, and nitrofurantoin. Susceptibilities from the last urinary isolate can be used to guide prophylaxis. A major limitation of prophylaxis is the selection of drug-resistant organism, a common problem in these patients. One study showed that infection by a multidrug-resistant bacteria significantly increased the risk of recurrent UTIs [74]. A reduction in immunosuppression, if possible, and optimal control of other variables such as diabetes mellitus may also help. A summary of microbial etiology and management of various forms of urinary infection is provided in Table 12-1.

12.7 Candiduria

Candiduria is defined as the presence of $>10^5$ Candida organisms in mid-stream urine. There is no clear consensus on whether all candiduria in the kidney transplant setting should be treated. Safdar et al. [75] reviewed the epidemiology of candiduria in 192 renal transplant recipients. Predictors of candiduria in this population were similar to those in the general population and included female sex, intensive care unit admission, antibiotic use, and diabetes. Candiduria was

TABLE 12-1. Suggested management of various clinical presentations of urinary tract infection in renal transplant recipients

Clinical presentation	Microbial etiology	Suggested management
Symptomatic cystitis	Enterobacteriaceae, *Enterococcus* sp., staphylococci, *Pseudomonas* sp., *Candida* sp.	Empiric oral therapy: first line: ciprofloxacin 500 mg PO b.i.d. ± amoxicillin 500 mg PO tid. Then directed therapy once culture results available. Treatment duration 5–7 days
Allograft pyelonephritis	As above, if culture is negative, consider unusual causes—e.g., adenovirus, mycoplasma (see "Sterile pyuria" row below)	Empiric therapy with IV or oral antibiotics as above. Directed therapy once culture results are available. Treatment duration 2–4 weeks
Recurrent UTI	As above	Rule out structural causes or persistent focus of infection. Consider oral antibiotic prophylaxis and re-evaluate in 6 months
Asymptomatic bacteriuria	As above	No need for empiric therapy. Await culture and susceptibility for directed therapy. Repeat culture to rule out contamination. Treat if stent is present or within 6 months posttransplant or persistent bacteriuria with same organism
Sterile pyuria	*Mycobacterium* sp., *Chlamydia trachomatis, Ureaplasma urealyticum*, fungi	Urine culture for acid-fast bacilli, fungi, other special testing as indicated
Yeast	*Candida* sp., unusual causes—*Malassezia* sp., *Trichosporon* sp.	Remove risk factors (urinary catheter, broad-spectrum antibiotics), rule out fungal bezoar by imaging, repeat urine culture, if symptomatic or persistent funguria, treat with fluconazole 400 mg daily. If fails to eradicate, then speciate and do susceptibility testing. Avoid amphotericin products

associated with decreased survival, likely reflecting severity of illness; however, therapy of asymptomatic candiduria was not associated with increased survival. On the other hand, candiduria may be a marker of fungal aggregates along the urinary tract, which can cause obstruction [76–78]. An attempt should be made to reduce risk factors such as removal of urinary catheters or avoidance of broad-spectrum antibiotic therapy. Fluconazole can be used as empiric therapy although persistent candiduria should lead to abdominal imaging to rule out a persisting source and removal of urinary catheter if present. If funguria persists, the yeast should be speciated and undergo susceptibility testing. If the isolate is found to be fluconazole resistant, therapy can be escalated to expanded spectrum azoles or echinocandins depending on susceptibility patterns.

12.8 Graft-Site Candidiasis

In a large review of 18,617 kidney transplants, the incidence of graft-site candidiasis was 1 per 1000 [79]. The majority of these infections have occurred in the first 3 months posttransplant. Many of these infections involved a fungal arte rial aneurysm. Usually, these are secondary to a single *Candida* sp. (primarily *C. albicans*) although bacterial coinfection has been found. Over 20 cases of fungal arterial aneurysm have been described in the literature from 1972 to 2015 [80–82]. In several, but not all, cases, *Candida* sp. was also recovered from organ preservation fluid. The significance of recovering *Candida* sp. from graft preservation fluid is unclear. Matignon et al. [83] have shown that of eight kidneys transplanted where preservation fluid was infected with *Candida* sp., none developed arterial aneurysm after 1–2 years of follow-up. Albano et al. [79] reviewed the cases of graft-site candidiasis in renal transplant centers in France from 1997 to 2005. Of the 18 cases found, 13 were due to *C. albicans* and others due to other *Candida* sp. Although most cases were that of fungal arteritis, infected urinoma, graft-site abscess, and surgical site infection also occurred. Treatment of fungal arteritis consists of antifungals and surgical ligation of the external iliac artery. Transplant nephrectomy is required in 50–70 % of cases and death has occurred in 17–50 % of cases especially where diagnosis is delayed. This is a serious complication of transplantation and important to recognize since massive bleeding can quickly lead to death.

12.9 Cytomegalovirus

CMV remains one of the most common opportunistic infections post kidney transplantation. While CMV is discussed in detail in Chap. 23, there are several important aspects unique to kidney transplantation. CMV reactivates in up to 50 % of renal transplant recipients depending on other risk factors such as donor/recipient serostatus, use of prophylaxis, and type of immunosuppression [84, 85]. In the current era, reactivation of CMV after renal transplantation most commonly presents as detection of asymptomatic viremia. In patients who present with symptoms, the majority has a flu-like illness with one or more of fever, malaise, and myalgias termed "CMV syndrome." CMV may also cause end-organ disease including enteritis, hepatitis, and pneumonitis, and rarely allograft nephritis. CMV has also been shown to have "indirect" or "immunomodulatory" effects in the transplant population. In the renal transplant setting, CMV has been associated with acute kidney rejection although the association of CMV with chronic allograft dysfunction is less certain [86–89]. A study comparing CMV prophylaxis

versus preemptive therapy demonstrated improvement in long-term graft survival with the use of prophylaxis [90].

The greatest risk of reactivation is in patients who are seronegative but receive an organ from a seropositive donor (D+/R−). For this group, universal prophylaxis with antivirals for 3–6 months posttransplant has been suggested [85]. The majority of large randomized controlled trials have either had a majority of kidney recipients or included only kidney recipients. In many instances, these results have been extrapolated to other transplant populations. The IMPACT trial compared 3 months with 6 months of valganciclovir prophylaxis in 319 D+/R− kidney transplant recipients. The incidence of CMV disease in the two arms was 36.8 % versus 16.1 %, respectively [91]. Longer term follow-up of these patients did not reveal an increased incidence of late onset CMV disease beyond the first year posttransplant in the group that received 6 months prophylaxis [92]. Routine viral load monitoring for CMV after the prophylaxis period is employed by some centers although its utility is unknown [93, 94]. Other tools such as cell-mediated immunity assessment may be of better utility for predicting late-onset CMV disease [95]. Various regimens are available for prophylaxis and include oral valganciclovir, oral ganciclovir, and valacyclovir [96, 97]. However oral ganciclovir is no longer available in many jurisdictions. Valacyclovir prophylaxis has only been extensively studied in the renal transplant population and appears to be effective [98]. Use of valacyclovir in D+/R− patients was also associated with a significant reduction in acute rejection episodes [98] but this finding has not been replicated in more recent studies [91]. Recipients that are seropositive are also at risk especially when antithymocyte globulin preparations are used for induction immunosuppressive therapy. These patients are either given antiviral prophylaxis for the first 3–6 months posttransplant or monitored at regular intervals with molecular assays (pre-emptive therapy) [85].

Treatment of CMV consists of induction doses of intravenous ganciclovir 5 mg/kg b.i.d. or oral valganciclovir 900 mg b.i.d. until viremia is at a low or undetectable level. Thereafter, maintenance doses can be used. In a randomized, multicenter study of intravenous ganciclovir versus oral valganciclovir for CMV disease, success rates were not significantly different, and current recommendations suggest that oral therapy can be used first line for mild to moderate CMV disease [84, 99]. It is worth noting that the majority of patients included in randomized treatment study were renal transplant recipients. In addition, long-term clinical or virologic recurrences were not significantly different between groups [100].

12.10 Polyomavirus

Polyomavirus-associated nephropathy (PVAN) is an important cause of graft dysfunction and graft failure. The incidence of PVAN ranges from 1 to 10 %; the majority of infections are due to BK virus-associated nephropathy (BKVAN) and very rarely PVAN may be due to JC virus alone [101]. In the modern immunosuppressive era, BK virus is one of the most important causes of infections after kidney transplantation and is discussed fully in Chap. 30. The pathogenesis, epidemiology, and management are briefly described in the following text.

After primary infection, the virus establishes latency primarily in the urogenital tract including renal cortex, medulla, urothelial cells, and bladder. The majority of viral reactivation occurs in the first year posttransplant. Reactivation of polyomavirus in the ureter can lead to stenosis whereas bladder reactivation can manifest as hemorrhagic cystitis. However, both of these are uncommon complications after kidney transplantation. Reactivation, replication, and inflammation within the kidney result in BKVAN. Usually, the only clinical manifestation is a rise in serum creatinine. A definitive diagnosis of BKVAN is made by kidney biopsy that demonstrates varying degrees of inflammation and/or fibrosis, often with intranuclear viral inclusions. Immunohistochemical staining using antibody directed against the SV40 T antigen or VP capsid proteins shows a characteristic nuclear staining reaction. Since disease may be patchy, a biopsy may occasionally be false negative.

Various risk factors for BKVAN have been suggested in several studies [102–106]. These include human leukocyte antigen (HLA) mismatches, history of acute rejection and use of antilymphocyte therapy, recipient age > 55, and recipient seronegativity. However, a large study of 1001 renal transplant recipients, 4 % of whom developed BKVAN, did not find any specific risk factors [107]. Recently, the use of more aggressive immunosuppression protocols such as with ABO incompatible transplants have been associated with a higher risk for BKVAN [108]. In another study, BK viremia was associated with the use of tacrolimus-mycophenolic acid combination versus cyclosporine-based immunosuppression [109].

The cornerstone of therapy for BKVAN is reduction in immunosuppression. All other options are less well studied and randomized controlled trials are lacking. Cidofovir, which is a nucleotide analogue of cytosine, has activity against DNA viruses. Results of case reports and series with cidofovir are difficult to interpret due to the concomitant decrease in immunosuppression [110]. Brincidofovir is a lipid conjugated oral formulation with decreased toxicity and appears to have activity against BK virus [111]. Further studies are ongoing. Leflunomide also appears to have antiviral properties in addition to its immunosuppressive action. Josephson et al. [112] showed stabilization of renal function in the majority of patients with BKVAN treated with leflunomide [112]. However, in a study of 52 patients treated with leflunomide, there was no association with viremia clearance and no correlation between serum concentrations of its metabolite A77 1726 and clearance [113]. Although leflunomide has been used as a treatment option, its adverse effects include hemolysis, transaminitis, and pancytopenia. Other experimental therapies that have been attempted or proposed

include fluoroquinolones, intravenous immunoglobulin (IVIg), and rituximab [114]. However in a recent randomized trial of 3 months of levofloxacin versus placebo for prevention of BK viruria and viremia, no benefit was demonstrated [115]. In addition, in a placebo controlled trial for treatment of BK viremia, in 39 patients, no beneficial effort of levofloxacin was observed [116]. Finally, the risk of recurrence after retransplantation for graft loss secondary to BKVAN does not appear to be increased [117].

It is well established that BK viruria and viremia are a prerequisite for histologically proven BKVAN. Given the lack of specific treatment for BKVAN and the high incidence of graft loss, routine screening for BK virus for early detection in the first year posttransplant is now recommended by most authorities [118]. Screening may be done by NAT testing of urine or plasma/blood. Detection of virus in the urine in itself has poor predictive value for BKVAN, but should trigger testing in blood or plasma. Detection of viremia is a better predictor of BKVAN and early intervention with judicious lowering of immunosuppression prevents the development of BKVAN.

12.11 Other Viral Infections Post Kidney Transplant

12.11.1 Adenovirus

Adenoviruses are non-enveloped DNA viruses with at least 52 known serotypes that are capable of causing a variety of illness in immunocompetent and immunocompromised hosts [119]. This includes upper and lower respiratory tract infection, conjunctivitis, keratoconjunctivitis and pharyngoconjunctival fever, hepatitis, and disseminated disease. Although adenovirus disease may manifest with these clinical syndromes in kidney transplant patients, several cases of adenovirus-related disseminated disease, pyelonephritis and hemorrhagic cystitis have also been described [120, 121]. Hofland et al. [122] reviewed 37 cases of adenovirus hemorrhagic cystitis in kidney transplant patients. All cases occurred within the first year posttransplant and the majority presented with fever and dysuria and hematuria. Graft dysfunction was present in the majority of patients and viral changes or acute rejection may be seen in kidney biopsies. Adenovirus species B predominates with serotypes 7, 11, 34, 35 causing most of the diseases. Diagnosis can be made by indirect methods such as serology or methods that directly demonstrate the presence of virus such as plasma polymerase chain reaction (PCR) and culture. In situ hybridization, immunohistochemistry, or PCR of fixed tissue can also identify adenovirus. Routine monitoring for adenovirus is not beneficial. In a surveillance study using blood PCR for adenovirus, it was found that self-limited adenoviremia can occur in 7 % of solid organ transplant patients with 58 % being asymptomatic [123]. There is no specific therapy for adenovirus, although clinical studies have focused on cidofovir and ribavirin. As discussed above, brincidofovir is a new oral lipid-conjugated cidofovir that has in vitro activity against many DNA viruses including adenovirus, and may be an option in the future. Immune reconstitution plays an important role in the clearance of adenovirus; therefore, decreasing doses of immunosuppressive medication is important.

12.11.2 Parvovirus B19

Parvovirus is a single-stranded DNA virus of the genus *Erythrovirus*. Although most infections are nonspecific flu-like illnesses, specific clinical syndromes have been described. In children, parvovirus infection is termed “fifth disease” that causes a facial rash resembling “slapped cheeks”; adults with parvovirus can develop a polyarthropathy syndrome; the virus can also lead to transient aplastic crisis in those with chronic hemolytic anemia and hydrops fetalis leading to intrauterine fetal death in pregnant women. Infection in transplant recipients is unlike that of immunocompetent patients in that viral replication can persist for prolonged periods of time [124]. Recurrent parvovirus infections have also been described [125]. Onset of parvovirus-associated syndromes can occur at any time posttransplant and has been described as early as 2 weeks. One study of 60 adult kidney transplant patients showed a 10 % rate of parvovirus viremia in the first year posttransplant [126]. The mode of acquisition of the virus is unknown in renal transplant recipients. Possibilities include inhalation of infected aerosols as in the immunocompetent host but also transmission from the donor. The possibility of viral reactivation also exists such as in the case of herpesviruses although little is known about parvovirus latency or cellular reservoirs. Parvovirus has well-established association with hematologic abnormalities including pure red cell aplasia and acute or chronic anemia in kidney transplant recipients. Since anemia is such a common problem in renal transplant recipients, it is important that physicians keep this diagnosis in mind especially for cases of severe, unexplained, or recalcitrant anemia. In one series, 3 out of 8 (38 %) of renal transplant patients with erythropoietin-resistant anemia (Hgb < 10 g/dL) were parvovirus positive by qualitative plasma PCR [127]. Other cell lineages may also be affected and lead to leucopenia and thrombocytopenia. Less well-developed associations exist with transient allograft dysfunction, collapsing glomerulopathy, acute rejection, and thrombotic microangiopathy. Other associations in renal transplant recipients have also been described such as hepatitis, encephalitis, and cerebral angiitis. Serologic studies have limited utility since they can be hampered by transfusion or immunoglobulin therapy. In addition, transplant recipients may not mount an antibody response. Instead, direct detection of virus by qualitative or quantitative DNA PCR is the most useful method. There is no specific antiviral therapy for parvovirus infection although

various management options have been suggested. These consist of a decrease in immunosuppression and/or IVIg. Various dose regimens of IVIg have been used and range from 0.4 to 1 g/kg for 4–10 days.

12.11.3 West Nile Virus

WNV is a flavivirus that has established itself in North America. WNV is most commonly transmitted via mosquito bites but can also be transmitted through blood transfusion and organ donation. Several series of WNV infection transmitted from infected donors to recipients have now been described with the majority of recipients developing encephalitis [128–132]. Donor screening with WNV NAT has been instituted in most organ procurement organizations to reduce the risk of transmission. Donor screening is usually done during periods of high WNV activity or year-round. Community-acquired cases also continue to occur and WNV encephalitis has been described in several kidney and kidney–pancreas transplant recipients [133–136]. A seroprevalence study in organ transplant recipients estimated the risk of neurologic disease to be 40 % compared to <1 % in immunocompetent hosts [137]. Diagnosis of WNV is based on an appropriate clinical picture, a lymphocytic pleocytosis in the cerebrospinal fluid (CSF), and WNV IgM in CSF and serum. A salient feature in transplant recipients is the absence of IgM or delayed positivity. In these cases WNV NAT may be used for diagnosis. There is no specific antiviral therapy for WNV although in the majority of the described cases, immunosuppression was significantly reduced. The successful use of WNV hyperimmune globulin obtained from healthy Israeli blood donors has been described for a liver transplant recipient who developed donor-derived WNV [138]. In addition, IVIg has been successfully used for transplant recipients with WNV [139]. Some studies suggest benefit with ribavirin or interferon-α but this has not been specifically studied in the transplant setting [140, 141]. As a result, many transplant programs advise patients to use personal protection measures such as longsleeved clothing, insect repellent containing *N*,*N*-diethyl-metatoluamide (DEET), and avoidance of outdoor activity at dusk and dawn, a time when mosquitoes are most active.

12.12 Kidney Transplantation in the HIV-Positive Recipient

Traditionally, infection with the HIV was considered to be a contraindication to transplant. However, in the last two decades, the increasing use of HAART (highly active antiretroviral therapy) has significantly increased the life span of HIV-infected individuals [142]. Recent estimates indicate up to 2–17 % of HIV-positive patients have chronic renal disease although rates vary significantly worldwide [143]. A major cause of end-stage renal disease in this population is HIV-associated nephropathy (HIVAN), which is a collapsing glomerulopathy that is more common in African Americans with HIV as well as focal segmental glomerulosclerosis. HIV itself may be a cause of IgA nephropathy. In addition, glomerulonephritis associated with HBV and HCV can also occur in coinfected patients. End-stage renal disease can also be compounded by toxicities of antiretrovirals such as indinavir, tenofovir, and ritonavir.

Recent studies have shown that both graft and patient survival of HIV-infected patients undergoing kidney transplant are similar to HIV-negative patients [144]. However, HIV-infected patients who are coinfected with HCV have significantly lower 5- and 10-year graft and patient survival than HIV-negative/HCV-positive patients [144, 145]. Over time, kidney transplant outcomes for HIV-infected patients have improved [146]. Using the Scientific Registry of Transplant Recipients data from 2003 to 2011, Locke et al. determined that HIV+ patients have a twofold greater risk of acute rejection compared to the HIV-negative group; however, HIV+ patients that received antithymocyte globulin induction had 2.6-fold lower rejection rates than those that received no induction [147]. Acute rejection rates have ranged between 13 and 50 % of patients likely due to variability in patient selection and posttransplant induction and maintenance immunosuppressives [148, 149]. HIV viremia has generally been well controlled. Most centers performing transplants in HIV-positive setting have carefully selected patients for transplant based on CD4 counts, undetectable viral load, and lack of significant opportunistic disease including progressive multifocal encephalopathy, CNS lymphoma, chronic intestinal cryptosporidiosis, and visceral Kaposi's sarcoma [149]. In addition, HIV genotypic and phenotypic testing predictive of suppression on HAART therapy as well as patient compliance are important factors in selection [150].

Posttransplant, drug interactions between immunosuppressives and anti-retrovirals need to be considered. Maintenance immunosuppression consists of steroids, calcineurin inhibitors (CNIs), and MMF. Although both CNIs can be used, patients on tacrolimus had lower rejection rates [149]. There is significant interaction between CNIs and protease inhibitors that inhibit the cytochrome P450-3A system. In this case, CNI doses need to be reduced appropriately. Conversely, CNI doses need to be increased with non-nucleoside reverse transcriptase inhibitors (NNRTIs) such as efavirenz that induce cytochrome P450-3A. Nonetheless, frequent measurement of levels is required to reach the optimal dose. The management of adverse events such as bone marrow suppression is also complicated since HAART, transplant immunosuppressives, and prophylactic antimicrobials (e.g., TMP/SMX, valganciclovir) can be myelosuppressive.

Preventative strategies post kidney transplant in the HIV patient are similar to those in the HIV-negative transplant population with some exceptions. Antipneumocystis pro-

phylaxis is usually given life long rather than the 6- to 12-month prophylaxis regimen used by many centers. TMP/SMX is the standard prophylaxis with alternatives such as dapsone and atovaquone for TMP/SMX intolerance or allergy. Patients with very low CD4 counts may require prophylaxis for other organisms such as mycobacterium avium complex and toxoplasmosis.

Recently, transplantation of kidneys from HIV+ donors into HIV+ recipients has been studied in South Africa where the availability of dialysis is limited [151–153]. In their cohort of 27 transplants, Muller et al. have found 74 % and 84 % patient and graft survival, respectively, at 5-years. At 3-years, the rejection rate was 22 % and HIV viral loads remained undetectable. In the United States, approval of the HIV Organ Policy Equity (HOPE) act in 2013 will allow for increasing research in this emerging area [154]. The field of transplanting HIV-positive patients is relatively young. Changes in selection of patients, optimal immunosuppression regimens, and knowledge of posttransplant infections will increase as results of ongoing studies become available.

12.13 Infectious Risks of Transplant Tourism

Given the limited supply of deceased donor kidneys, a significant proportion of patients from Western countries travel to Eastern countries where kidney transplantation can be performed on a pay basis. Often this involves the illegal trafficking of organs [155]. In this setting, a kidney is harvested from a live donor who needs money to support his/her family or repay debt. The transplant is performed for cash and the patient returns home to be managed by his/her transplant physician. Unfortunately, the standards for organ procurement do not necessarily meet those of registered transplant centers worldwide. Often the patient returns home with no or minimal medical records. Basic information such as donor CMV, EBV, and hepatitis serologies may be unknown. There is an increased incidence of postoperative complications including wound infections, perinephric abscess, colonization, and infection with multidrug-resistant organisms including extended-spectrum beta-lactamase-producing gram-negative bacteria [156–159]. Other issues, although less common, may include malaria (donor-derived or community acquired), donor-derived TB, fungal infections, acquisition of HIV, and hepatitis. To address the issue of organ trafficking and transplant tourism, an international consensus took place in Istanbul that outlined the strategies needed to increase donation and ensure safety of living donors [160]. Physicians caring for individuals who have received transplants in this manner should be aware of the potential exposures.

12.14 Antimicrobials and Nephrotoxicity

Given the unique susceptibility of the allograft to a number of insults, it is reasonable to avoid antimicrobial agents with a high risk of nephrotoxicity. Additive or synergistic nephrotoxicity can occur with antimicrobials and immunosuppressive drugs, especially CNIs. Specific agents that can cause nephrotoxicity include aminoglycosides (e.g., gentamicin, tobramycin, amikacin), intravenous colistin, and standard amphotericin B as well as lipid amphotericin preparations. Routine use of these agents should be avoided especially if an alternative antimicrobial agent can be used. Consideration should be given to the risks and benefits when using these agents with careful monitoring of renal function and drug levels when possible. Other agents such as vancomycin, high-dose TMP/SMX, and high-dose quinolones may be nephrotoxic when combined with potentially nephrotoxic immune suppressants. When possible, antimicrobial levels should be monitored.

12.15 Posttransplant Vaccinations

Vaccinations in renal transplant recipients follow the guidelines for vaccinations in all solid organ transplant recipients [19]. Both inactivated and live vaccines (such as those for VZV (Varivax; Zostavax)) can be given prior to transplant. If the opportunity exists, a vaccine is likely more effective if given pretransplant as early as possible in the course of progressive renal disease. If a live vaccine is given pretransplant, one should wait approximately 4 weeks before transplantation to avoid vaccine-related disease. In the posttransplant period, vaccinations generally begin no sooner than 3–6 months. Although vaccinations could be administered earlier, there are limited data regarding immunogenicity. Yearly influenza vaccine is recommended for all transplant patients. There is no evidence for a link between vaccination and allograft rejection. Renal transplant recipients appear to have a reasonable humoral response to a single dose of influenza vaccine whereas double dose vaccine does not appear to be beneficial [115]. One study showed better graft survival in patients who received influenza vaccine in the first year [161]. Family members and household contacts of the transplant patient should also be vaccinated with annual influenza vaccine. Pneumococcal vaccine is also recommended for renal transplant recipients. In a randomized trial of renal transplant recipients, the pneumococcal conjugate vaccine had an increased trend to greater humoral responses compared to the polysaccharide vaccine [162]. There was a significant decline in titers in the same cohort followed for 3 years with either vaccine [163]. Therefore, most vaccine authorities now recommend one dose of conjugate vaccine followed by one dose of polysaccharide vaccine with a minimum interval of 8 weeks [164]. Other inactivated vaccines generally follow the guidelines for non-transplanted individuals.

12.16 Pneumocystis Prophylaxis

Antimicrobial prophylaxis is recommended post kidney transplant, although there is a wide variety of practices [165]. Prophylaxis for *P. jirovecii* pneumonia (PCP) is generally instituted in the early posttransplant period. PCP appears to be more common in renal transplant recipients who have undergone treatment for multiple rejection episodes and received polyclonal/ monoclonal antibodies [166]. Corticosteroid use has classically been associated with the occurrence of PCP. However, anti- B-cell therapies such as rituximab for the management of antibody-mediated rejection in this population may also increase risk of PCP [164]. PCP prophylaxis is generally instituted for the first 6 months to 1 year posttransplant. Consideration can be given to continuation or reinstitution of prophylaxis beyond this time if the patient remains on high-dose corticosteroids or receives monoclonal antibodies for rejection. In the past 5 years, clusters of late PCP infections have been reported by some investigators suggesting re-emergence of this pathogen [167–169]. The primary agent for prophylaxis is TMP/SMX. Doses used are one single-strength tablet once daily or one double-strength tablet thrice a week. However, a proportion of patients will have toxicity such as leucopenia, rash, and drug-induced hepatitis. In addition, higher doses of TMP/SMX can lead to renal dysfunction. Alternatives to TMP/SMX are once monthly inhaled pentamidine, oral dapsone, or atovaquone [170].

12.17 Summary

In summary, infectious complications continue to be an important cause of morbidity and graft dysfunction in kidney transplantation. With evolving immunosuppression regimens, infectious etiologies are also changing. A prime example of this is BK nephropathy, which has emerged as an important cause of graft loss only in the era of more modern immunosuppression. Traditional infections associated with kidney transplantation, such as CMV, also pose challenges but modern management strategies have reduced the burden of such infections significantly. UTIs and related bacterial infections are very common in these patients and this is an area where clinical trials are needed to better define appropriate therapeutic strategies.

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13
Risks and Epidemiology of Infections After Pancreas or Kidney–Pancreas Transplantation

Atul Humar, Roberto Lopez, and Abhinav Humar

After experiencing continuous improved rates and growth in total numbers in the 1980s and 1990s, pancreas transplants have suffered a steady decline in procedures performed and new listings in the last decade [1]. Despite this, they still represent the most reliable way to consistently achieve euglycemia for insulin-dependent diabetics, with islet transplantation alone as a promising alternative [2]. For a diabetic patient who has progressed to end stage renal disease (ESRD), the treatment of choice is a pancreas transplant (in conjunction with a kidney transplant). Dramatic improvements in outcomes have resulted from refinements in surgical technique, superior immunosuppressive regimens, and a better understanding of donor and recipient risk factors. By 2010, nearly 36,000 pancreas (1 AG) transplants had been performed worldwide; however, the number of transplants in the USA has steadily decreased from 2000 performed in 2004, to almost a 50 % decline in 2013 when just over a 1000 cases were reported [3], despite the increase in half-life for an SPK pancreatic graft in the last 14 years [4, 5].

The causes for this decrease are uncertain, but likely can be attributed to better glycemic controls with current insulin regimens, worsening in the donor quality due to the increased prevalence of obesity, or to more stringent controls in outcome translating into an increased selectivity in donors and recipients for individual transplant centers.

Yet, despite the improvements, the incidence of complications remains high after a pancreas transplant. In fact, the surgical complication rate is the highest of all routinely performed solid organ transplants [6]. Technical failure is the most common cause of pancreas graft loss in the first-year posttransplant, most notably manifesting as vascular thrombosis in half of the cases of technical graft loss or infections in close to one-fifth of them [7].

Pancreas recipients are at significant risk for infectious complications posttransplant, which can lead to significant morbidity and mortality. Numerous risk factors include long-standing diabetes with multi-organ involvement (especially renal failure), which can lead to an immunosuppressed state even before any immunosuppressive drugs are begun, impaired tissue healing, and poor vascular flow. The donor pancreas graft contains a hollow viscera (i.e., the duodenum) that is contaminated and is thus a potential source of infective organisms. The transplant surgery itself involves opening non-sterile viscera in the recipient (i.e., either the bladder or the small bowel), with potential for further contamination. Posttransplant, higher levels of immunosuppression is generally required than other solid organ transplants. Moreover, pancreas recipients have an increased incidence of acute rejection, which requires bolus doses of immunosuppressive drugs. All of these factors combine to explain why pancreas recipients have the highest incidence of infections of all solid organ transplant recipients [8].

13.1 Spectrum and Classification of Infections

The spectrum of possible infections after a pancreas transplant is wide. No completely satisfactory classification system exists for the types of infections seen in pancreas recipients. They may occur in the early (0–30 days), intermediate (30–180 days), or late (beyond 180 days) posttransplant period [9]. They may be related directly to the surgical procedure or to some complication that develops afterwards or may be opportunistic, resulting from the recipient's overall immunosuppressed state (Table 13-1).

Classification by pathogen into bacterial, viral, or fungal infections is not always clinically useful. One type of pathogen may be involved in several different types of infections, or a number of different pathogens may be involved in a single infection (e.g., an intra-abdominal abscess with several different bacterial and fungal pathogens). Classification by timing posttransplant, into early, intermediate or late infections, has some merit, because many infections follow a typical temporal pattern. Again, however, this information may not be very useful when making clinical decisions about treatment.

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Table 13-1. Summary of infections after pancreas transplant, risk factors, and methods of prevention

Infection	Risk factors	Preventive measures
Surgical		
Intra-abdominal infections	• Older donor age • Donor obesity • Prolonged cold ischemia • Recipient obesity • Prolonged pretransplant peritoneal dialysis • Graft pancreatitis posttransplant • Duodenal leak posttransplant	• Careful donor selection • Minimize cold ischemia • Prompt diagnosis and treatment of duodenal leaks
Duodenal leaks	• Prolonged cold ischemia • Duodenal devascularization during preparation of graft • Impaired bladder emptying in recipient • Late CMV infections of transplant	• Minimize cold ischemia • Careful attention to donor duodenum blood supply • Pretransplant bladder assessment in recipient
Recurrent UTI	• Bladder-drained graft • Neuropathic bladder • Foreign body exposed in bladder	• Enteric drainage of graft if possible • Oversewing of exposed staple lines in bladder
Wound infections	• Longer operative procedures • Intraoperative duodenal spillage • Recipient obesity • Intra-abdominal infection	• Prophylactic antibiotics preoperatively • Minimize recipient risk factors
Medical		
Cytomegalovirus	• Pretransplant serostatus (D+/R− at highest risk; also frequent in D+/R+ and D−/R+ groups) • Degree of immunosuppression; especially use of antilymphocyte therapy	• Multiple strategies, but usually universal prophylaxis to all at-risk patients • Usually oral valganciclovir for 3–6 months • Longer duration prophylaxis preferred for high-risk patients (e.g., 6 months)
EBV-related posttransplant lymphoproliferative disease	• Intensity of immunosuppression (especially antilymphocyte therapy) • Pretransplant EBV serology (EBV D+/R− at highest risk)	• Uncontrolled studies suggest antiviral prophylaxis may be of benefit • Strategies to prevent CMV disease may also reduce EBV-related disease • Minimize immunosuppression • Avoid antilymphocyte therapy • Consider monitoring viral loads with preemptive changes to immunosuppression in response to increasing viral loads

Classification by the primary method of treatment, into surgical and medical infections, is useful in a broad sense. Surgical infections are those that require some invasive intervention as an integral part of their treatment. These types of infections generally occur soon after the transplant operation itself and are usually related directly to it or to some complication occurring as a result of the operative procedure. Surgical infections are less likely to be related to the recipient's overall immunosuppressed state, though obviously this plays some role. Typical examples of surgical infections include generalized peritonitis, intra-abdominal abscesses, wound infections, and, in bladder-drained pancreas grafts, recurrent UTIs.

In contrast, medical infections are primarily treated with antiviral, antibacterial, or antifungal agents. They tend to occur in intermediate and late posttransplant stage and are usually related to the recipient's overall immunosuppressive state [9]. Typical examples include infections secondary to cytomegalovirus (CMV), polyomavirus-induced nephropathy, pneumonias, and Epstein–Barr virus (EBV)-related posttransplant lymphoproliferative disorder (PTLD).

13.2 Transplant Procedure

A brief review of the surgical procedure itself is necessary in order to better understand the infections (especially surgical infections) that may develop in these pancreas recipients (see Figures 13-1 and 13-2).

Pancreas transplants are performed in insulin-dependent diabetics who may or may not have coexisting kidney failure from diabetic nephropathy. Most pancreas transplants in the USA are in diabetics with ESRD who also require a kidney transplant [5]. The two organs may be transplanted either

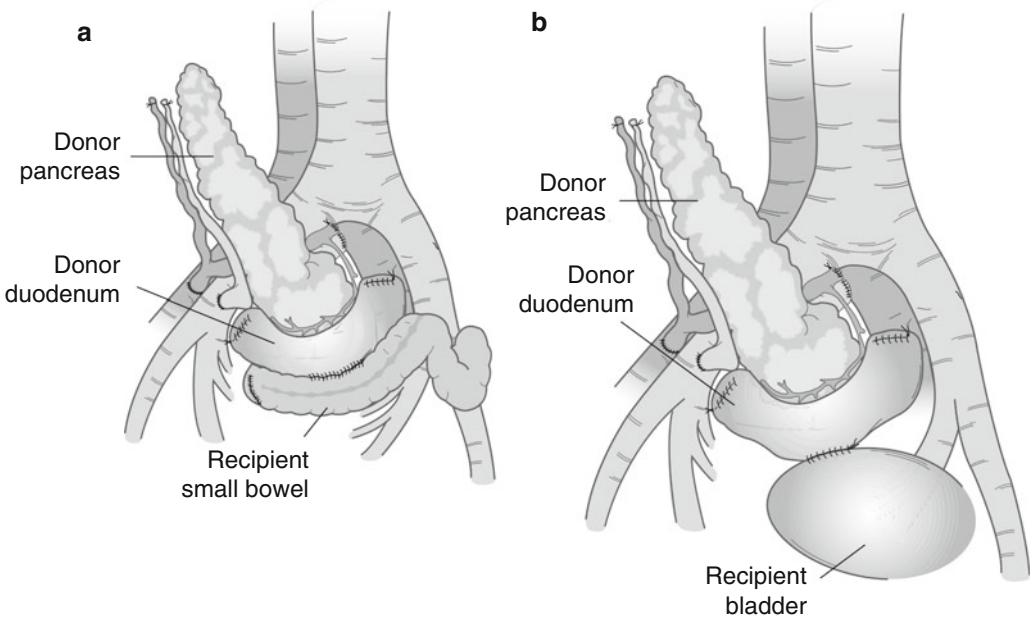

FIGURE 13-1. (**a**) Pancreas transplant with enteric drainage of the pancreas. (**b**) Pancreas transplant alone with bladder drainage of the pancreas.

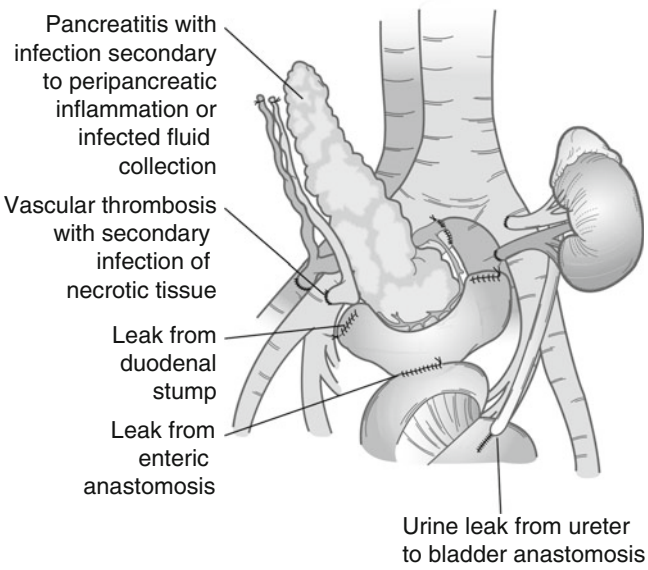

FIGURE 13-2. Potential sources of surgical infections after simultaneous pancreas–kidney transplant.

simultaneously (SPK—simultaneous pancreas–kidney) or sequentially: first the kidney transplant, followed at a later date by the pancreas (PAK—pancreas after kidney). For diabetic patients who retain kidney function, a pancreas transplant alone (PTA) may be indicated. Overall, SPK transplantations accounted for approximately 72% of total pancreas transplants in 2010, PAK for 17%, and PTA for 7% [1].

Ideal PTA candidates include those with widely fluctuating glucose levels, hypoglycemic unawareness, frequent insulin reactions, or recurrent episodes of diabetic ketoacidosis. These three pancreas recipient categories—SPK, PAK, PTA—do not differ with regard to the possible infections that may result. Rather, as discussed later, the relative risk for certain infections may differ in the three groups.

Donor operation [10]: A pancreas from a deceased donor is removed en bloc with the duodenum and spleen. Many centers routinely flush the donor duodenum with some antibiotic solution to decrease the degree of contamination, but there is no good proof that doing so decreases the risk of subsequent intra-abdominal or wound infections [11]. Before the pancreas is implanted in the recipient, it is generally prepared on the "back table." A donor splenectomy is performed, the donor duodenum is trimmed, and any vessels that may potentially bleed are oversewn or ligated. Careful preparation of the pancreas can help decrease the risk of subsequent intra-abdominal infections. Trauma to the pancreas can lead to graft pancreatitis, a risk factor for intra-abdominal infections [12]. Similarly, large hematomas from unligated vessels may predispose to intra-abdominal infections.

Recipient operation [13]: Whether the pancreas transplant is performed by itself or in conjunction with a kidney transplant, the pancreas graft is placed in an intraperitoneal position. This placement is important to keep in mind, as it influences the area of spread of subsequent infections originating around the graft itself. Usually, the pancreas graft is placed in the right lower quadrant, with inflow and outflow

of blood achieved by anastomosing the graft vessels to the recipient iliac artery and vein. Another option is to anastomose the venous outflow of the graft to the recipient mesenteric vein, thus draining the endocrine secretions (i.e., insulin) into the portal (instead of systemic) circulation. The exocrine secretions (i.e., amylase) of the pancreas can be drained either into the bladder (BD—bladder-drained) or into the small bowel (ED—enteric-drained) (Figure 13-1). The method of drainage has important implications for complications that may occur, especially graft duodenal leaks. ED is more physiologic, but a duodenal leak with ED grafts may lead to more devastating infections than seen with BD grafts. If a leak occurs with a BD graft, urine leaks within the peritoneal cavity; with ED grafts, enteric contents leak into the abdominal cavity. The choice of ED or BD varies with the clinical situation and with the individual center's preference. BD is associated with increased urologic complications (such as recurrent urinary tract infections (UTIs)) but makes monitoring the function of the graft easier (by measuring the amount of amylase in the urine) [14]; however, reliance solely on clinical parameters such as hyperglycemia, serum amylase and lipase, C-peptide levels, hemoglobin-A1C, or (if bladder-drained) urinary amylase are insufficient because they are either too late or too nonspecific, risking a graft rejection going undetected, which is associated with graft failure [15, 16]. ED does away with the urologic complications, but may be associated with more severe intra-abdominal infections. Most centers now generally drain all pancreas grafts into the small intestine. In 2010, ED was used in 91 % of SPK, 89 % of PAK, and 85 % of PTA patients [17]. Our preference is to wait until after reperfusion of the pancreas before making the final decision. If there is evidence of significant reperfusion injury of the graft with reperfusion pancreatitis or poor perfusion of the duodenum, it is generally safer to drain the graft into the bladder.

13.3 Risk Factors for Infections

The risk factors for infections in pancreas recipients depend on the site and type of infection. For example, the factors predisposing to wound infections posttransplant are completely different from those associated with CMV infections. In general, risk factors can be classified into those present in the recipient pretransplant, those related to the donor, those related to the recipient intraoperatively, and those that occur posttransplant.

Pretransplant latent infections or infectious exposures can reactivate or worsen early posttransplant, once heavy-dose immunosuppression is initiated. Pretransplant immunity, or lack of immunity, to certain viral pathogens can be an especially important risk factor for posttransplant infections. For example, recipients seronegative for CMV or EBV have a high incidence of posttransplant infections with these viruses, especially if the donor was seropositive [18, 19]. The recipient's overall medical status may play an important role in posttransplant infections. All pancreas recipients have long-standing diabetes as a risk factor. In addition, some may have these additional risk factors as well: poor nutritional status (especially those who have brittle diabetes or who have been on dialysis long term); advanced peripheral vascular disease; or frequent hospitalizations pretransplant. Recipient obesity is a well-known risk factor for posttransplant infectious complications, especially involving the wound [20].

Donor factors may play an important role in patients at risk for posttransplant infections. Transmission of infections from the donor, especially bacterial, is very uncommon [21]. But viruses such as CMV, EBV, hepatitis B or C, and HIV can certainly be transmitted to any recipient who has not had previous exposure to them [22]. Besides direct donor transmission, other donor variables may also affect posttransplant infections in the recipient. Older donor age (i.e., ≥45 years old) and donor obesity are both associated with increased risk for posttransplant complications such as pancreatitis and graft thrombosis, which can lead to intra-abdominal abscesses or wound infections [23, 24].

Intraoperative risk factors for infections include a longer operative procedure with significant bleeding, prolonged cold, and warm ischemia of the graft, and the SPK category of transplant type. Grafts with prolonged ischemia times are more prone to develop pancreatitis, duodenal leaks, and thrombotic complications—all of which may predispose to intra-abdominal infections and various other infectious complications [25]. SPK recipients generally have a higher incidence of intra-abdominal and wound infections, as compared with PAK or PTA recipients [5, 8]. The possible reasons are that SPK recipients undergo a longer operative procedure (with more extensive dissection required), are often sicker at the time of the transplant, their poor nutritional status, and the fact that they receive not one but two transplanted organs that may develop complications.

Posttransplant risk factors for infection are generally related either to the development of posttransplant complications or to the level of immunosuppression. Almost any complication that requires a reoperation will predispose to wound infections [26]. Complications such as pancreatitis or vascular thrombosis of the graft can result in fluid collections or in devitalized, necrotic tissue, which can become secondarily infected. Clinically significant peripancreatic fluid collections (PPFCs) can be seen in 16 % of pancreas transplants, and are usually seen within 3 months after transplantation. Sometimes, they can be resolved by radiological drainage though often they will require laparotomy [27]. The strongest risk factor for intra-abdominal infections posttransplant is a leak from the duodenal cuff or anastomosis [28]. The level of immunosuppression is an important risk factor, especially for opportunistic infections. The higher the level of immunosuppression, the greater the risk. Long induction regimens involving powerful antilymphocyte agents or bolus antirejection treatment have been clearly identified as risk factors for a variety of infections [28, 29].

13.4 Immunosuppression

As mentioned earlier, higher levels of immunosuppression are generally associated with a higher likelihood of infection, especially with opportunistic viral and fungal pathogens. The amount of immunosuppression given to an individual recipient is usually related to the immunologic risk. PTA recipients generally have a higher risk of rejection than do SPK or PAK recipients, and therefore they tend to receive higher levels of immunosuppression [30]. The risk of rejection in all three pancreas recipient categories is highest early after the transplant; hence, most centers use induction therapy immediately posttransplant, generally an antilymphocyte intravenous agent such as anti-thymocyte globulin alemtuzumab, or basiliximab [31, 32]; the use of OKT3 for induction therapy has fallen out of favor over the past couple of decades due to the concern of possible increased PTLD risk [33]. After induction therapy, recipients are maintained on some combination of antirejection agents, usually a calcineurin inhibitor (cyclosporine or tacrolimus), an antimetabolite (mycophenolate mofetil or azathioprine), and prednisone. Newer, more powerful agents are becoming available that more specifically inhibit the immunogenic response. There is concern, however, that higher level of immunosuppression seen with such agents may increase the risk of infection.

The level of maintenance immunosuppression may be dramatically increased if the recipient requires short-term treatment for acute rejection. High-dose steroids or antilymphocyte preparations may be used. Infections may then develop.

13.5 Intra-abdominal Infections

Of the infections that may develop after pancreas transplant, intra-abdominal infections are generally the most serious, and most likely to be life threatening. They may range from diffuse peritonitis to localized abscesses. Their presentation, management, and clinical course will, in part, depend on their location and on the recipient's overall condition.

The incidence of intra-abdominal infections in this population has steadily decreased over time. During the 1980s, the reported incidence was very high, close to 30 % [34]. The high incidence of mortality or graft loss in pancreas recipients who developed intra-abdominal infections was also very high. By the 1990s, the incidence of intra-abdominal infections had decreased to 15–20 % [35]. By 2000, the incidence of major intra-abdominal infections had decreased to 5–10 % [36]. At present, this incidence has remained relatively stable [37]. The exact reason for this lower incidence is likely multifactorial. Better surgical technique, better preservation methods, and better immunosuppressive regimens (resulting in a lower incidence of acute rejection) have all likely played a role.

Although the incidence has decreased over time, intra-abdominal infections continue to be a major problem in pancreas recipients and are still a common technical reason for graft loss (other causes being vascular thrombosis, pancreatitis, and hemorrhagic pancreatitis) [38, 39]. Some of these infections probably originate because of contamination from the donor duodenum, which is opened during the transplant procedure. Some correlation has been shown between organisms identified from cultures of the donor duodenum and later cultures from the intra-abdominal infection [40].

Duodenal leaks (DL) are probably the most significant risk factor for intra-abdominal infections, especially with ED grafts; in one recent study, DLs were accountable for a quarter of all 1-year graft losses [41]. But, donor factors also play an important role in one multivariate analysis of risk factors for intra-abdominal infections, older donor age was found to be a strong risk factor for intra-abdominal infections [36]: if the pancreas donor was older than 40 years, the incidence of intra-abdominal infections was 16.2 %; if younger than 40 years, 2.9 % ($P=0.009$). Donor obesity is another factor predisposing the recipient to infectious complications. In one analysis, if the donor body mass index (BMI) was >25 kg/m^2, the incidence of intra-abdominal infections was 11.7 %; if the donor BMI was >30 kg/m^2, the incidence was 21.0 % ($P=0.07$) [43]. Often, the pancreas from obese donors has a significant amount of extra fatty tissue on the graft. This fat may become necrotic after the transplant and serve as a nidus for infection. Recipient risk factors for the development of intra-abdominal infections include obesity and the presence of occlusive peripheral vascular disease. This is an important issue given that the US donor population is becoming increasingly old, obese, and diabetic and only approximately 15 % of US deceased donors in 2013 donated a pancreas for transplantation [42, 43].

Graft pancreatitis developing early posttransplant may also lead to infection. Pancreatitis and hemorrhagic pancreatitis are important causes of technical failure of the pancreas graft [39]. Graft inflammation and swelling early posttransplant is likely a manifestation of reperfusion injury after cold ischemia. Inflammation of the graft or the surrounding peripancreatic tissue can lead to fluid collections around the graft and often to subsequent infections. Necrotic pancreatic tissue from severe pancreatitis can become secondarily infected; this almost always requires removal of the graft to manage adequately.

Other risk factors for intra-abdominal infections include the pancreas recipient category and the type and duration of dialysis pretransplant. Prolonged pretransplant dialysis, especially peritoneal dialysis, is associated with a higher incidence of posttransplant infections [44]. As with infections in general, SPK recipients have a slightly higher incidence of intra-abdominal infections than do PAK or PTA recipients [5].

The clinical presentation of intra-abdominal infections will depend on their severity and location. Generalized peritonitis is usually associated with some catastrophic event, such as graft duodenal leak with spillage of enteric contents or urine into the peritoneal cavity. It may also occur as a result of perforation of some other viscus, unrelated to the transplant (e.g., perforated gastric ulcer, perforated cecum). Generalized peritonitis is diagnosed clinically; the physical exam is the most helpful tool. Such patients appear ill, with tachycardia, elevated temperature, falling blood pressure, and diffuse tenderness with guarding on palpation of the abdomen. A plain film or CT of the abdomen is not usually necessary, but may demonstrate free air. Treatment involves prompt return of the recipient to the operating room to determine the reason for the peritonitis and will often depend on the degree of contamination. If a duodenal leak is identified and the spillage is of brief duration and localized, then a primary repair of the duodenal stump or anastomosis, followed by copious lavage of the abdomen, often has a good chance for success. However, if significant inflammation and contamination is noted in and around the pancreas, the graft should probably be removed, to adequately deal with the inciting process. Again, depending on the degree of contamination, the recipient may need to return to the operating room on several occasions, so that the abdomen can be adequately "washed-out."

Fortunately, most intra-abdominal infections do not fall into the generalized peritonitis category. Instead, most of them consist of localized fluid collections in and around the pancreas graft. Presentation is usually systemic, with symptoms such as fever, nausea, vomiting, or abdominal distention, with localized pain and guarding over the region of the fluid collection. A CT scan with contrast is the best diagnostic tool in this clinical situation.

About half of these localized abscesses are monomicrobial; common isolates include *Enterococcus*, *Escherichia coli*, *Klebsiella*, and *Pseudomonas* [45]. The others tend to be polymicrobial, containing two or more bacteria or both bacterial and fungal species. The most common fungal species isolated was *Candida albicans*, but recently *Candida kruzei* and *Candida glabrata* have been increasing in incidence (likely related to the widespread use of agents such as fluconazole for prophylaxis). One benefit of routine prophylaxis has been that the incidence of intra-abdominal infections with fungal pathogens has significantly decreased [36].

Treatment of localized intra-abdominal infections involves adequate drainage and appropriate antimicrobial or antifungal coverage. These infections can often be drained percutaneously under radiologic guidance, at least as a good initial approach. But if the infected fluid is not adequately drained or if the recipient does not improve clinically, a laparotomy should be performed to achieve adequate drainage of all infected fluid. The rate of early laparotomy for first time pancreas transplantation has been reported be close to 30 % in pancreas transplant recipients [46]. If the pancreas graft is obviously involved with the infection or appears tenuous with areas of necrosis, a graft pancreatectomy should be performed. Or, if despite adequate drainage of infected fluid, the recipient continues to deteriorate, then the graft should be removed (in case there is infection within the graft itself).

Intra-abdominal infections, especially those that are fungal in origin, can sometimes lead to uncommon complications, such as mycotic pseudoaneurysms of the iliac artery [47]. Usually, such pseudoaneurysms are at the site of the anastomosis of the "Y-graft" to the recipient iliac artery, but other locations are possible. These pseudoaneurysms may rupture into the abdominal cavity, causing hypotension, abdominal pain, and distention. Or, they may rupture into the pancreas graft, bladder, or small bowel, causing massive hematuria or gastrointestinal bleeding. Treatment in the case of rupture requires emergency laparotomy, and usually, removal of the graft and reconstruction of the artery. Usually, this reconstruction requires use of autogenous tissue such as saphenous vein because of the underlying infection.

The development of intra-abdominal infections after a pancreas transplant has a significant detrimental impact on patient and graft survival. Adequate treatment of these infections requires graft removal in 50–60 % of recipients—even more if the infections are polymicrobial with fungus present [45]. In one series of 213 pancreas recipients, graft survival at 1-year posttransplant was 82 % in those without an intra-abdominal infection vs. 60 % in those with ($P=0.01$) [12]. The mortality rate associated with intra-abdominal infections ranges from 6 to 20 % [45]. Again, higher mortality rates are associated with polymicrobial and fungal infections.

13.6 Duodenal Leaks

Leaking from the donor duodenum may occur either at the site of anastomosis (to the bladder or bowel) or at the duodenal stumps. A leak results in spillage of pancreatic enzymes as well as either enteric contents (with ED) or urine (with BD). In either case, there is spillage of contaminated contents, resulting in either a localized or a generalized intra-abdominal infection.

The reported incidence of duodenal leaks is between 5 and 15 % [48, 49] and has not changed appreciably in the last 10–15 years [41]. Most leaks occur early posttransplant, usually in the first few weeks. Excluding a technical problem with the anastomosis or with the closure of the duodenal stumps, most leaks probably occur as a result of ischemia of the duodenum. Disruption of the blood supply to the duodenum, especially at its ends, may occur during the back-table preparation of the graft. Prolonged cold ischemia with subsequent ischemic reperfusion injury of the duodenum is a definite risk factor for a subsequent leak. In one analysis of 294 pancreas recipients, duodenal leaks developed in 23 (7.8 %) [25]. If cold ischemia time was <15 h, the incidence of leaks

was 1.4 %. But if cold ischemia was 15–20 h, the incidence increased to 5.8 %; 20–25 h, 14.1 %; and >25 h, 16.7 %.

Other risk factors for duodenal leaks include impaired wound healing caused by immunosuppressive therapy, most notably high-dose steroids or the newer immunosuppressive agents [50]. Other predisposing factors include bladder dysfunction with poor emptying, leading to high pressure within the bladder and to stress on the anastomosis of the BD graft [51].

Duodenal leaks may also occur late posttransplant. In contrast to early leaks, late leaks are usually not due to technical causes, but rather due to ischemia or infection. CMV infections leading to duodenal ulcers and perforation have been described [41, 50].

Clinical presentation will vary, depending on whether BD or ED was used. With ED, a leak usually presents with generalized peritonitis as enteric contents spill into the peritoneal cavity. With BD, symptoms are generally less severe; usually there is pain, often associated with elevated serum amylase level (both of which improve with placement of a Foley catheter).

Diagnosis is based on the clinical presentation and radiologic imaging. With ED grafts, a leak can be difficult to diagnose with current imaging techniques. A CT scan may show some free air and fluid in the peritoneal cavity, but these findings are not diagnostic. With BD grafts, however, a CT cystogram or a fluoroscopic cystogram are sensitive and specific.

Treatment of duodenal leaks may be either surgical or medical. With ED grafts, all such leaks require operative repair. If there is significant contamination and infection around the pancreas graft, a graft pancreatectomy should be performed. With BD grafts, leaks (especially small ones) may often be managed conservatively. A Foley catheter is inserted to decompress the bladder and left in place until imaging studies demonstrate no further leak. If catheter placement is not successful, or if the recipient is unwell clinically, then operative repair is necessary. It may involve either direct repair of the leak site with some form of patch (most commonly omentum) or conversion to ED. About 20 % of duodenal leaks with BD grafts require operative repair [50].

13.7 Recurrent Urinary Tract Infections

UTIs are very common after a pancreas transplant, especially with BD grafts. Common organisms include *Enterococcus*, *Candida*, and *Pseudomonas*. Several factors may predispose the recipient to recurrent UTIs. The defense mechanism of the bladder may be altered by pancreatic secretions, leading to an increased chance for infections. Other risk factors include a diabetic neuropathic bladder with incomplete emptying, alkalization of the urine from bicarbonate in pancreatic secretions, presence of a Foley catheter, and contamination from the transplanted duodenum. Lastly, foreign bodies in the bladder, in the form of staples or sutures at the anastomosis, may serve as a nidus for infection [52].

The incidence of recurrent UTIs in pancreas recipients ranges from 10 to 40 % [8, 53, 54]. The vast majority of these infections will respond to appropriate antibiotic therapy. If the infection is recurrent or if it does not respond to therapy, then cystoscopy should be performed. If foreign bodies, such as staples, are identified that may be serving as a nidus for infection, they should be removed. If UTIs still persist, the graft should be converted into ED.

13.7.1 Wound Infections

Infections involving the surgical wound are much more common after a pancreas transplant as compared with a kidney transplant alone. Most involve the layers of the abdominal wall superficial to the fascia. Sometimes, however, the fascia and muscle layers can be involved with a devastating, necrotizing type of infection. The reported incidence of wound infections is 7–35 % [8, 48, 55]. They may occur in isolation, but a significant number of them are associated with deep, intra-abdominal infections. In one series of pancreas recipients, 38 (18 %) had wound infections. Of these 38 recipients, 21 (10 %) had isolated involvement of the superficial wound and 17 (8 %) had a coexisting intra-abdominal infection [48].

Several factors account for the higher incidence of wound infections after a pancreas transplant. Most prominent is the fact that the donor duodenum is a contaminated hollow viscera; spillage from it while the anastomosis is being done can contaminate the surgical wound. With ED, enteric contents can spill onto the wound.

The typical pancreas transplant recipients' long-standing history of diabetes also plays a role; the general surgery literature shows diabetes to be a risk factor for wound infections [56]. Compared to kidney transplant recipients, these patients undergo a longer operative procedure, which is another risk factor. The even longer operative procedure that SPK (vs. PTA or PAK) recipients undergo is also associated with a higher wound infection rate [4]. Another significant risk factor for wound infections in pancreas recipients is obesity [43].

The pattern of microbial agents found in wound infections depends on the existence of any underlying intra-abdominal infection. An isolated wound infection is usually monomicrobial, with Gram-positive bacteria being most commonly involved. But with an underlying intra-abdominal infection, more than 50 % of such wound infections are polymicrobial and may involve fungi (most commonly *Candida* species).

Local signs and symptoms include wound pain, erythema, and drainage from the wound. Treatment consists of opening the wound, regularly changing dressing, and packing the wound. Antibiotics are used if there are systemic signs of infection or significant cellulitis around the opened wound.

A CT scan of the abdomen should be done to rule out an underlying intra-abdominal infection, especially in the presence of systemic symptoms.

13.8 Prophylaxis

Infection rates remain high after pancreas transplants [8] but they have decreased significantly in the last decade. One reason is the use of better prophylaxis. Most centers now use some form of routine antibacterial, antiviral, and antifungal prophylaxis for all pancreas recipients.

The main purpose of antibacterial prophylaxis is to diminish the likelihood of wound infections. Generally, a wide spectrum antibiotic is used to target the Gram-positive and Gram-negative organisms that may contaminate the wound at the time of surgery. Many centers also routinely use antifungal prophylaxis in all pancreas recipients. A typical regimen consists of copiously irrigating the abdominal cavity with amphotericin B solution, followed by 1 week of oral fluconazole immediately after surgery. The incidence of fungal intra-abdominal infections has decreased significantly with use of routine prophylaxis [36]. Given the generally poor outcomes with fungal intra-abdominal infections, some form of antifungal prophylaxis is warranted.

Other preventative regimens include ganciclovir for CMV prophylaxis (for 3–6 months posttransplant, or for 6 weeks after bolus antirejection treatment) and trimethoprim/sulfamethoxazole for *Pneumocystis* prophylaxis.

13.9 Cytomegalovirus Infections

13.9.1 Epidemiology

Pancreas recipients are generally considered to be at high risk for CMV infection and disease. In addition to the standard risk factors such as pretransplant CMV serostatus (the highest risk being donor positive and recipient negative or D+/R− [57, 58]) several factors are more specific to pancreas transplants. The result may be higher rates of CMV infection and disease compared to some other solid organ recipients. Pancreas recipients often require higher levels of immunosuppression than do other solid organ recipients. They may also have an increased incidence of acute rejection, which requires more aggressive immunosuppressive therapy—specifically antilymphocyte products, known to be a major risk factor for CMV [57, 58]. Also, the amount of lymphoid tissue within the pancreatico-duodenal graft is quite high, probably serving as a source of donor-acquired latent CMV and resulting in a high viral inoculum into the recipient. Finally, many studies have reported a high rate of baseline recipient CMV seronegativity in pancreas recipients [59–62], which increases the number of CMV-mismatched (D+/R−) recipients.

In the absence of specific CMV prophylaxis, the rate of CMV disease is quite high. In one study of 34 pancreas recipients on quadruple immunosuppression with antilymphocyte therapy induction but no CMV-specific prophylaxis, 71 % of them developed CMV infection [59]. Of the total of 34 pancreas recipients in the study, 17 (50 %) developed symptomatic CMV disease, at a mean of 43 days (range, 15–63 days). Symptomatic CMV disease was most common in the CMV D+/R− group [59]. It was also frequent in the lower risk D+/R− and D+/R− groups and may also occur in D−/R− patients who receive unscreened or unfiltered blood products. As in other solid organ recipients, CMV disease may present in pancreas recipients as a viral syndrome or as tissue-invasive disease. Tissue-invasive disease is relatively common (although likely lower in the current era of prophylaxis, and early treatment) and may include hepatitis, pneumonitis, and gastrointestinal disease; it may also involve the pancreas graft, resulting in CMV pancreatitis, although the latter is relatively infrequent compared to other forms of tissue-invasive disease such as colitis [59, 62–64]. Disease rates in pancreas recipients on specific CMV prophylaxis are lower, and disease tends to occur later in the posttransplant period. Moreover, reported disease rates and presentations may differ from center to center, depending on immunosuppressive regimens and on the type and duration of CMV prophylaxis.

13.9.2 CMV Prevention

Given the high rates of CMV infection and disease in pancreas recipients, most centers use some form of universal antiviral prophylaxis. Generally, prophylaxis is given to all “at-risk” patients (donor or recipient CMV seropositive). Randomized controlled trials of CMV prophylaxis in pancreas recipients are scarce, and most studies are retrospective, single-center reviews or are trials involving predominantly kidney transplant recipients with a small number of pancreas transplant patients included.

A number of agents are available for CMV prophylaxis but oral valganciclovir is likely the best option in these patients primarily based on data from large randomized trials in other groups of organ transplant recipients. In a large international trial comparing 3 months of valganciclovir prophylaxis vs. oral ganciclovir prophylaxis for D+/R− patients, both regimens had similar efficacy [65]. However, only a small number of pancreas transplant recipients were included in this trial. Nonetheless, these results have been supported by smaller nonrandomized trials. Oral ganciclovir is currently no longer available in many jurisdictions. Prior to ganciclovir, acyclovir-based prophylaxis was commonly used. However, the use of acyclovir as CMV prophylaxis is no longer recommended [57].

The major problem with prophylaxis in pancreas transplant recipients appears to be the high incidence of late-onset

CMV disease [66]. That is, CMV disease occurs after the discontinuation of prophylaxis and early diagnosis may be missed if patients are no longer followed as closely by their primary transplant program. Fallatah et al. reviewed 130 pancreas transplant recipients who received antiviral prophylaxis [67]. Despite prophylaxis the incidence of CMV infection or disease was 24 % and was especially high in the D+/R− cohort (44 %). The median duration of prophylaxis was 104 days CMV infection/disease occurred a median of 34 days following discontinuation of prophylaxis. In a review of 252 pancreas transplant recipients (the majority of whom received antiviral prophylaxis) by Parsaik et al. [62], the cumulative incidence of CMV infection/disease was 15 % at 1 year and again was significantly higher in the D+/R− group. The majority of infections occurred within 1 year of transplant although late infections were not infrequent. The risk of late CMV disease appears to be higher as compared with kidney recipients and liver recipients on similar prophylactic regimens. In one study of 240 solid organ recipients given 3 months of oral ganciclovir, late CMV disease occurred in significantly more D+/R− SPK recipients (42 %) as compared with D+/R− kidney recipients (10 %) and D+/R− liver recipients (7 %) [68].

The occurrence of late-onset CMV disease has led many centers to prolong antiviral prophylaxis beyond the standard 3 months, especially in D+/R− patients [69]. In a study of 326 D+/R− kidney transplant recipients, patients were randomized to receive either 200 days vs. 100 days of valganciclovir prophylaxis [69]. The incidence of symptomatic CMV disease by 12 months posttransplant was 16.1 % vs. 36.8 % in the two arms, respectively (p =<0.0001). Although this trial did not include pancreas transplant recipients, a reasonable strategy in D+/R− pancreas transplant recipients would be to prolong prophylaxis to 6 months. Another approach to prevention is preemptive therapy. In uncontrolled trials, preemptive antiviral therapy (generally with oral valganciclovir or intravenous ganciclovir) in recipients given antilymphocyte therapy appears to be of some benefit [70, 71]. More commonly, preemptive therapy refers to s virologic monitoring (e.g., by PCR) with subsequent preemptive antiviral therapy [72, 73]. This approach has its advantages and disadvantages compared to prophylaxis but has not been well studied in pancreas transplant recipients. Commonly many centers will use a hybrid approach in which frequent virologic monitoring is performed following a period of prophylaxis [57]. Again, the benefit of such a strategy has not been well evaluated in pancreas transplant recipients.

In summary, CMV prophylaxis is generally recommended for all pancreas recipients at risk for CMV disease. Oral valganciclovir for 3–6 months is a reasonable strategy, with the 6-month duration likely preferable in those who are D+/R− or who have recurrent rejection or high intensity of immunosuppression.

13.9.3 Management

Treatment of active CMV disease in pancreas and other solid organ transplant recipients is similar. Initial therapy is generally started with either intravenous ganciclovir or oral valganciclovir. In a randomized trial comparing 3 weeks of intravenous therapy to oral valganciclovir in 321 patients, both regimens had similar rates of viral clearance [63]. In patients who are very ill, or in whom gastrointestinal absorption may be impaired, initial intravenous therapy is preferred. The duration of initial therapy is usually 2–4 weeks, but it should be based, in part, on clinical and virologic responses. Pancreas recipients may have a very high rate of CMV disease recurrence. In one study of 332 cases of CMV disease in kidney and SPK recipients, CMV disease recurred after an initial course of treatment in 31 % of them (all were initially treated with 2 weeks of intravenous ganciclovir, followed by 10 weeks of oral acyclovir) [74]. However, the recurrence rate was significantly higher in SPK recipients (39.8 %) than in kidney recipients (20.5 %). According to a multivariate analysis, this increased rate of recurrence was primarily attributed to increased treatment of acute rejection episodes.

Based on the high risk of recurrence, a reasonable strategy is to give secondary prophylaxis with oral valganciclovir, after initial treatment therapy, especially in recipients with recent or recurrent episodes of acute rejection. With the widespread use of prophylaxis, ganciclovir resistance may be a problem in SPK recipients. Oral ganciclovir, in particular, has a low bioavailability (6–9 %) and therefore may promote ganciclovir resistance in heavily immunosuppressed patients. In one review of 240 liver, kidney, and pancreas recipients, all on 100 days of oral ganciclovir prophylaxis for CMV, ganciclovir-resistant CMV disease was observed in 5 (7 %) of 67 CMV D+/R− recipients [68]. Of these five recipients, four were pancreas recipients. Thus, the rate of ganciclovir resistance in D+/R− pancreas recipients with CMV disease in that study was 21 % (4 of 19) and was attributed to high levels of immunosuppression. The use of high than normal doses of intravenous ganciclovir or foscarnet may be required to treat ganciclovir-resistant CMV disease. The ongoing development of investigational anti-CMV compounds including brincidofovir, and letermovir, may ultimately prove useful in this setting [75, 76].

13.10 Epstein–Barr Virus and Posttransplant Lymphoproliferative Disease

EBV is associated with most cases of PTLD [77, 78]. The epidemiology of PTLD may vary from center to center, again depending on immunosuppressive regimens and other factors.

The largest single center study to date on pancreas recipients and PTLD retrospectively reviewed a single center experience in 787 pancreas and 569 kidney–pancreas recipients [79]. The 5-year cumulative incidence of PTLD ranged from 1.0 to 2.5 % with a trend towards increased rates in more recent times. PTLD tended to be more aggressive in pancreas recipients compared with liver or kidney recipients, with multiple sites involved at the time of presentation. A significant number of patients were noted to have T-cell lesions.

A recent study evaluated the UNOS database of pancreas transplants to identify factors that predicted PTLD [80]. In a total of 4205 pancreas transplants, the incidence of PTLD was 1.0 % (43 patients). In a multivariate analysis, recipient EBV seronegativity, and use of an immunosuppression regimen that did not include tacrolimus were among the risk factors associated with the development of PTLD.

An additional risk factor for PTLD is the intensity of immunosuppression, particularly the use of antilymphocyte products [77]. In an analysis of French registry data [81] which gathered data on the 10 year cumulative incidence of PTLD in kidney transplant recipients, SPK recipients had a higher likelihood of developing PTLD (adjusted hazard ratio 2.52). Induction therapy, and EBV mismatch were also identified as risk factors. Meador et al. [82] reviewed their cases of PTLD in pancreas recipients over a 6-year period. Of 337 pancreas recipients, 8 (2.4 %) developed PTLD, including 5 (2.8 %) of 179 SPK recipients, 1 (0.9 %) of 106 PAK recipients, and 2 (3.8 %) of 52 PTA recipients. PTLD occurred a mean of 137 days posttransplant (range, 34–348 days). PTLD involved the pancreas graft alone in three recipients, the graft and extra graft tissue in three, and extra graft tissue alone in two. Histologically, the tumors ranged from polyclonal B-cell hyperplasia to B-cell lymphoma. On ultrasound and CT imaging, five of the eight recipients had a diffusely enlarged pancreas graft, a finding that may be radiographically indistinguishable from acute rejection or pancreatitis.

In a study of 52 cases of PTLD in pancreas recipients that included data from the Israel Penn International Transplant Tumor Registry, the mean time to diagnosis was 18.9 months (range, 0.9–91 months). Sites involved were the lymph nodes alone (15.3 %), the lymph nodes plus other sites (38.4 %), the pancreas or kidney graft (27 %), the central nervous system (19 %), the liver (19 %), the gastrointestinal tract (17 %), and the spleen (11.5 %). The overall mortality was 61 %; those with central nervous system disease had a worse survival [83].

The available data on PTLD in pancreas recipients can be summarized as follows: The incidence of PTLD in most settings is likely in the range of 1–2.5 %. Most early cases are EBV-related B-cell PTLD. The most important risk factor likely relates to the EBV D+/R− transplant in which primary infection occurs in the absence of preexisting host immunity. The intensity of immunosuppression, and specifically the use of antilymphocyte products, is a major risk factor for the development of PTLD (as in other solid organ recipients). The broad pathologic and clinical spectrum of PTLD is similar to that seen in other solid organ recipients. However, PTLD commonly involves the pancreas graft, where it may result in a diffuse infiltrative pattern or in focal masses. Uncontrolled studies suggest that the use of antiviral prophylaxis may have some beneficial effect for prevention of PTLD in pancreas recipients [84]. A case–control study has also suggested that ganciclovir, more than acyclovir, may be beneficial for prevention of PTLD [85]. Also, because antiviral therapy may lead to a reduction in CMV infection (an independent risk factor for PTLD) it may indirectly have a beneficial effect on EBV and PTLD [86]. A potential approach is to measure EBV viral loads in high-risk patients (EBV D+/R−), with subsequent reductions in immunosuppression in patients with high or rising viral loads [78]. This strategy has not been well studied in pancreas transplant recipients. Once PTLD has occurred, treatment strategies, as with other solid organ groups, include reduced immunosuppression, antiviral therapy, rituximab, or chemotherapy, but mortality rate remains relatively high [78].

13.11 Other Infections

Given their high levels of immunosuppression, pancreas recipients are at risk for infections caused by numerous other opportunistic and nonopportunistic pathogens, including other herpesviruses, community-acquired pneumonia organisms, and environmental fungi [60, 87, 88]. Infections may occur because of environmental exposure or reactivation of endogenous latent organisms. The timeline is similar to that observed in other solid organ transplant recipients [88] although epidemiologic studies are lacking.

In the last decade, polyomavirus nephropathy or BK virus nephropathy (BKVAN) has been recognized as an important problem in kidney recipients. Large epidemiologic studies of polyomavirus infection of the kidney graft in SPK or PAK recipients are few in number and are generally retrospective. In a review of 146 SPK recipients, Lipshutz et al. [89] identified nine patients with BKVAN (calculated incidence 6.2 %), an average of 359 days posttransplant. In this cohort, BKVAN was the leading cause of renal graft loss in the first 2 years posttransplant. However, in another report, the incidence of BKVAN in 243 SPK recipients was 2.9 % with only three patients ultimately losing the kidney graft [90]. Pancreas graft involvement with polyomavirus has not been described. Interestingly, in one PTA recipient, polyomavirus nephropathy and kidney dysfunction in the native kidneys has been reported [91]. Current guidelines [92] for the prevention of BK nephropathy in kidney transplant patients should be followed for SPK patients and possibly for PAK patients who receive an augmentation in immunosuppression at the time of their pancreas transplant. This generally includes regular monitoring of plasma BK viral and reduction in immunosuppression in patients with high or rising viral loads.

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14
Risks and Epidemiology of Infections After Liver Transplantation

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Liver transplantation is now a widely accepted mode of therapy for patients with end-stage liver disease. Despite the improvement in surgical techniques, reduction in operative times, prolonged graft and patient survival, and progress in therapeutic treatment options over the last few decades, the rate of major infectious complications remains high in this population. Kusne et al. [1] and Paya et al. [2] reported rates of major infections of 67 % and 54 %, respectively, in their two series from two decades ago. More recent studies [3–5] did not show much improvement in the rate of infectious complications; the infection rate in liver transplantation is substantially higher than that of renal and heart transplant recipients [6] but lower than that of lung and small bowel transplant recipients [7, 8]. The main determinants of infection risk in this population include the mechanical aspects of the surgery itself, a myriad of host and donor factors [9], and the effects of the immunosuppression.

14.1 Infections Related to Mechanical Complications of Surgery

14.1.1 Early Postsurgical Infections

Liver transplantation is associated with a high rate of postsurgical infectious complications due to the technical complexity of the surgery and the frequent moribund state in which many of the recipients undergo the operation. Although the improved surgical techniques have resulted in shorter operative times, less blood product transfusions, reduced use of veno-venous bypass, and fewer biliary stents/T-tubes, the age and quality of the donors currently available for transplantation are much less favorable than the young trauma victims used most commonly previously [10]. The infections within the first month of transplant are largely due to bacterial and fungal pathogens. Although liver transplant recipients suffer from routine postoperative complications, such as pneumonia, urinary tract infections, and line sepsis, the most common sites of infection by far are intra-abdominal and the surgical wound [1, 11]. In a study by George et al. [11], one third of bacterial infections occurred in the first week after transplantation and more than one half within the first 2 weeks. The incidence of bacteremia ranges between 21 and 33 % [1, 2, 6]. The culprit organisms are usually enteric flora, including *Enterococcus* species and gram-negative *Enterobacteriaceae*, along with *Staphylococcus aureus* and coagulase-negative *Staphylococcus* [3, 12]. The rate of infection with antibiotic-resistant pathogens, such as vancomycin-resistant enterococcus (VRE) and methicillin-resistant *S. aureus* (MRSA), is high, as liver transplant candidates are often "antibiotic-experienced" with a history of frequent hospitalizations. A lengthy posttransplant intensive care unit (ICU) stay and spillage from the gastrointestinal (GI) tract at the time of biliary anastomosis exacerbate this problem.

Invasive fungal infections (IFIs) also occur most frequently in the first few weeks after surgery. The incidence of IFI after liver transplantation ranges from 5 to 42 % in various studies [1, 2, 12–16], with the case fatality ranging from 25 to 69 %. *Candida* species are the most frequent organism involved, followed by *Aspergillus* species. Abdominal wound infection by *Candida* is often a part of polymicrobial sepsis by enteric bacterial pathogens. Pathogenesis of abdominal IFI starts with the colonization of the GI tract with yeast, with the subsequent breach in mucosal integrity due to surgery, leading to contamination and the infection of the peritoneum. The presence of indwelling urinary and intravascular catheters facilitates the direct invasion of fungal organisms into the bloodstream.

The most consistently identified risk factors in the early posttransplantation period are the duration of surgery and retransplantation [1, 3, 11]. Figure 14-1 shows that the frequency of severe infection has an almost linear correlation with the total time spent in the operating room. Lengthy surgery presumably reflects technical difficulties and an attendant increase in stress from blood loss, hypothermia, tissue hypoperfusion, and prolonged exposure to microbiologic contaminants, whereas the tissue concentration of prophylactic antibiotic falls. The intraoperative transfusion requirement is closely related to infectious complications [3, 11–14, 17]. This is undoubtedly related to the duration and complexity of surgery and, more specifically, to the amount of

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intraoperative bleeding, which leads to tissue hypoxia and the formation of hematomas vulnerable to microbial seeding. Blood in the peritoneum also have an inhibitory effect on leukocytes [18]. There have also been studies showing that allogeneic blood transfusion causes downregulation of surveillance mechanisms of the immunity leading to tolerance and more infectious complications, which is not the case with autologous blood transfusion [19]. In addition to the length of surgery, the degree of early graft function can impact intraoperative blood loss, hemodynamic stability, and metabolic state (i.e., acidosis) in the recipient. Bacterial and fungal infections can be transmitted from the donor, both in the tissue and bile ducts/gallbladder. The presence of significant donor steatosis, advanced age, complications during organ procurement, and prolonged total ischemia time can exacerbate early graft function and set the stage for increased early posttransplant infection. In the past, the use of veno-venous bypass was common in an effort to stabilize the hemodynamic profile of the recipient during engraftment and as a tool to allow for additional time as needed to train surgical fellows in the techniques of the vascular anastomoses. Currently, due to the higher risks seen in our older and less high-quality donors, the additional time necessary to place a patient on bypass has essentially replaced its use to only very select cases. The surgical techniques most commonly used at present employ the "piggyback" technique that allows the retrohepatic inferior vena cava (IVC) to be left in place in the recipient (Figure 14-2). Instead, the right, middle, and left hepatic veins are combined into a single outflow tract or orifice upon which is attached the suprahepatic IVC from the donor liver graft, thus preventing the additional operating time, the hemodynamic instability of clamping the recipient IVC, and reducing the total number of vascular anastomoses to three rather than four. Unless the recipient has an anatomically abnormal distal common bile duct, current practice is to perform a duct-to-duct anastomosis. This reduces operative time, violation of the enteric system with inherent contamination, and additional enteric anastomoses that can contribute to increased postoperative ileus. Most transplant centers have abandoned the use of T-tubes (Figure 14-3), as their assessment of the quality of the bile produced early after transplant has greatly been replaced by other imaging and laboratory tools, thus reducing the 25 % incidence of leaks associated with these tubes and foreign body colonization of bacteria and fungi.

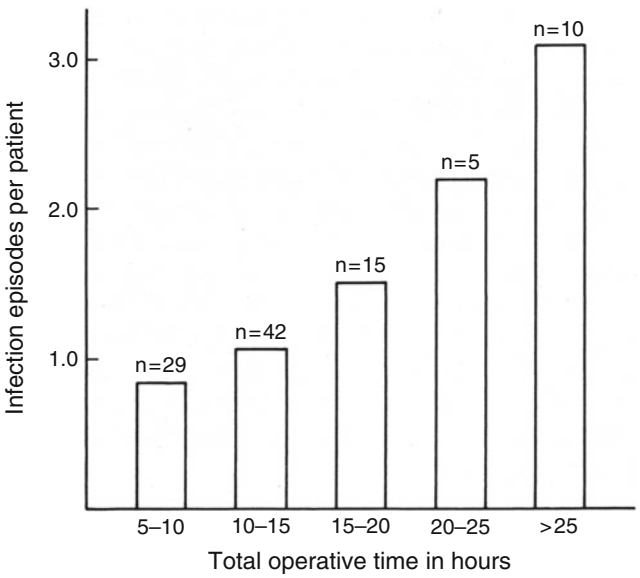

FIGURE 14-1. Frequency of severe infections in relation to the total operative time per patient in hours (Reproduced from Kusne S, Dummer JS, Singh N, et al. Infections after liver transplantation: an analysis of 101 consecutive cases. Medicine (Baltimore). 1988;67:132–143, with permission).

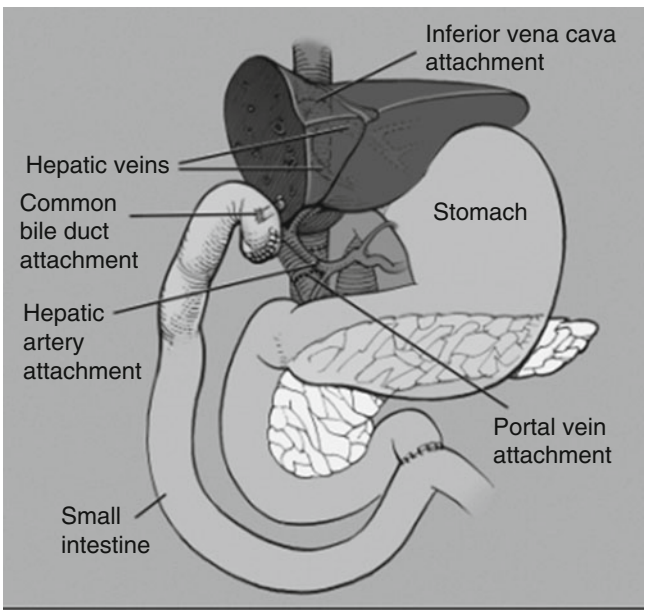

FIGURE 14-2. Living donor liver transplantation with piggyback caval technique allows recipient IVC to remain open and decreases abdominal vascular congestion, minimizing bacterial translocation from the gut. The recipient hepatic veins are clamped, leaving the IVC open, and a common orifice is created joining all three hepatic veins for anastomosis to the donor IVC. In this figure, only the hepatic vein was used to create the piggyback anastomosis (Reproduced from Marcos A, Fisher RA, Ham JM, et al. Right lobe living donor liver transplantation. Transplantation. 1999;68(6):798–803, with permission).

14.1.2 Mid-to-Late Postsurgical Infections

The usual options for biliary reconstruction during the transplant surgery are (a) end-to-end or side-to-side choledochocholedochostomy (CC) without a T-tube, (b) CC with a T-tube (Figure 14-3), or (c) Roux-en-Y choledochojejunostomy (CJ) (Figure 14-4). As previously mentioned, most practices currently employ CC without a T-tube unless the recipient has ana-

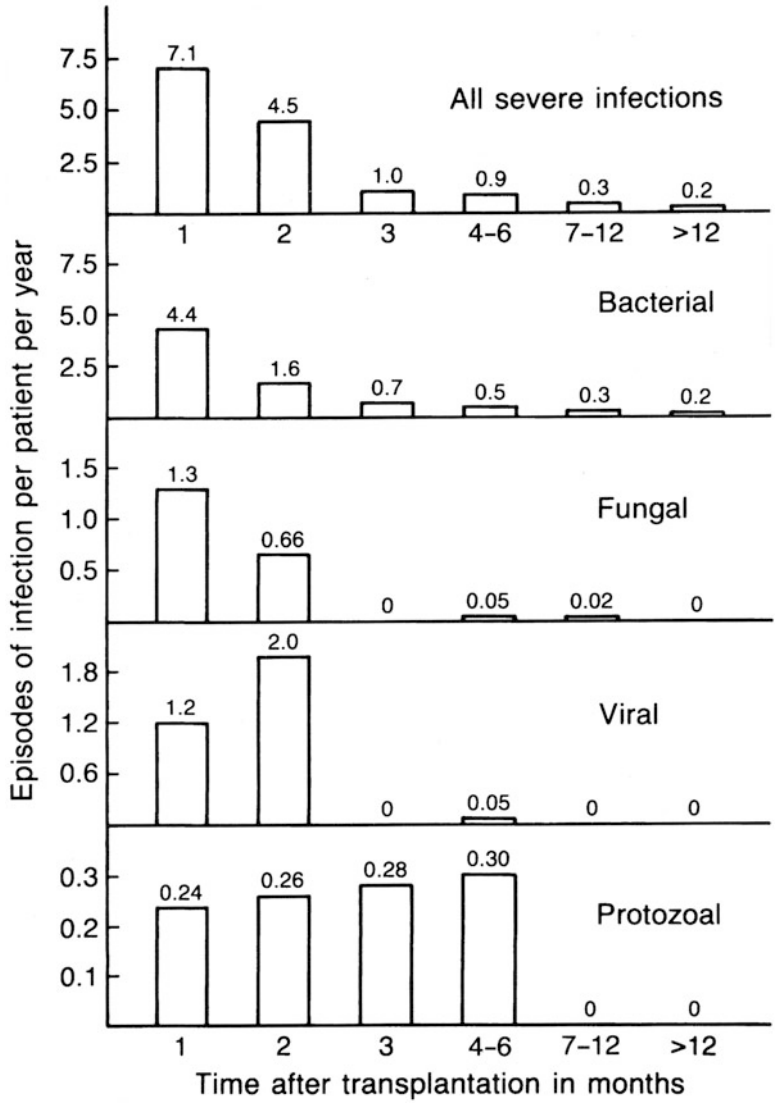

FIGURE 14-3. Classic liver transplant with full retrohepatic IVC and T-tube choledochocholedochostomy. Potential contamination with T-tube removal leading to bile leak (Reproduced from the Mayo Foundation, with permission).

tomically abnormal distal common bile duct, such as in patients with primary sclerosing cholangitis (PSC). In living donor liver transplantation (LDLT), most surgeons employ the use of direct duct-to-duct anastamoses, even in cases of multiple ducts, so that postoperative endoscopic studies may be more easily performed. For pediatric liver transplants and others where suitable recipient anatomy is unfavorable, Roux-en-Y hepaticojejunostomy (HJ) is preferred. Several studies suggest that patients who undergo CJ have a higher rate of early biliary complications, such as biliary stenosis, leaks, and infectious cholangitis, compared with those who undergo CC [1, 20]. Among the patients who have CC, those with T-tube placement have more biliary complications, including infections [21, 22]. On the other hand, the presence of the T-tube may delay the onset of biliary complications until after its removal, which usually occurs 2–3 months after transplantation. At that time, the immune system is not as suppressed as it is during the early postoperative phase [23]. The treatment of cholangitis involves the correction of the structural abnormality, such as strictures and leaks with percutaneous drainage and either percutaneous transhepatic or endoscopic stents, as well as administration of the appropriate antibiotic therapy for responsible organisms.

Hepatic artery thrombosis (HAT) is a major cause of morbidity and graft loss after liver transplantation. The reported incidence of HAT ranges from 1.6 to 20 % [24–26], with the higher incidence in children. According to the experience at the University of Pittsburgh, the overall incidence of HAT is 3.4 % in adults and 11.8 % in children [26]. In patients undergoing LDLT, HAT can occur in up to 20 % due to the greater technical difficulties facing the anastomosis of smaller vessels. HAT can present early (less than 7 days post-op) as arterial ischemia leading to fulminant

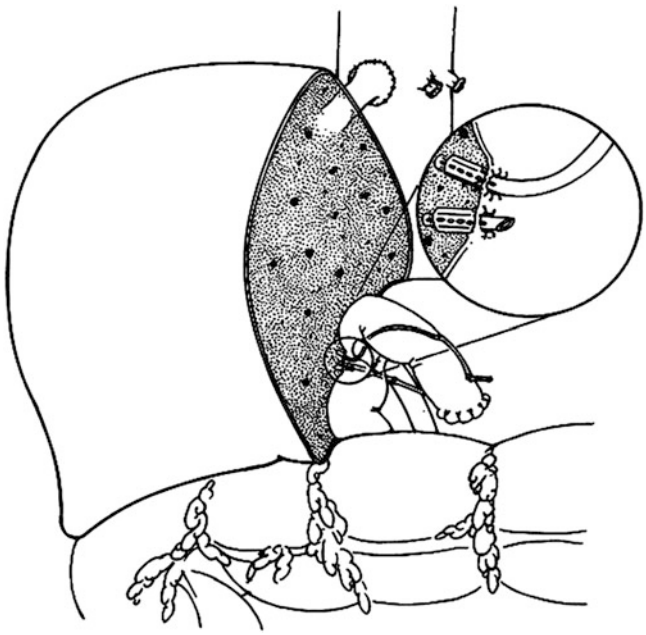

FIGURE 14-4. Living donor liver transplant with Roux-en-Y hepaticojejunostomy (HJ). Large cut surface of liver can lead to bile leaks and potential for intestinal content contamination from HJ leak (Reproduced from the Mayo Foundation, with permission).

hepatic failure. On the other hand, late HAT often presents insidiously, with impaired hepatobiliary perfusion leading to biliary necrosis with leaks, strictures, and, ultimately, allograft failure [6]. HAT is frequently associated with recurrent hepatic abscesses. Despite a combination of medical therapy and drainage of infected collections, the treatment of HAT-related hepatic abscesses is challenging, partly because of the difficulties in eradicating the infection in the absence of proper arterial circulation. Presentation with multiple small abscesses not amenable to drainage is common; in these cases, prolonged empiric antibiotic therapy with broad-spectrum enteric coverage is the therapy by default. According to one study [27], breakthrough bacteremia has been reported in 70 % of patients with HAT-related hepatic abscesses, with polymicrobial sepsis accounting for more than half of these cases. *Enterococcus* species and enteric gram-negative bacteria were the organisms most frequently isolated. Mortality was high in this group (37.5 %) regardless of the therapeutic approach. Revascularization surgery has been used with limited success [26]. In most cases, medical therapy is only a temporary solution until the patient can undergo retransplantation, which is considered the ultimate treatment for HAT and associated hepatic abscesses [24–27].

Liver abscess formation is unique to liver transplant recipients. Out of 2175 recipients of solid organ transplant from Cleveland Clinic Foundation between 1990 and 2000, 12 patients developed liver abscess and all of them were liver recipients [28]. The predisposing factors in eight (67 %) of them were HAT. Other predisposing factors may include hematomas, biliary complications, and, rarely, liver biopsy. Patients who have bacterial colonization of the biliary tree, such as those with biliary stents violating the sphincter of Oddi and recipients reconstructed with Rous-en-Y CC or CJ, are at increased risk for infection associated with liver biopsy and probably should receive prophylactic antibiotic therapy prior to and following the biopsy. Infected collections of bile are called by many authors "infected bilomas."

Infected bilomas or collections of bile inside or outside the liver may present like an intra- or perihepatic abscess and may need drainage and antibiotic treatment. Aggressive treatment of these bilomas, whether infected or not, is usually warranted as their presence in proximity to the hepatic artery frequently causes local inflammation and over time can lead to HAT. When bilomas are associated with HAT, retransplantation is usually needed. Diagnosis is usually made by CT or ultrasound. In one series from University of Wisconsin [29], 44 % of the patients presented with fever, 40 % had abdominal pain, and a third of the patients were asymptomatic; 79 % had liver enzyme abnormalities [29]. In that series the pathogens involved included *Enterococcus* (37 %), coagulase-negative *Staphylococcus* (26 %), *Candida* species (26 %), gram negatives (16 %), and anaerobes (5 %).

14.2 Host Factors of Infection

The underlying disease that caused the liver failure and other comorbid medical conditions contributes to the rate and type of infectious complications after transplantation. The most common causes of liver failure requiring transplantation are infection by hepatitis C virus (HCV) and hepatitis B virus (HBV), alcoholic liver disease, autoimmune hepatitis, PSC, and primary biliary cirrhosis (PBC). Patients with chronic viral hepatitides as the underlying disease are at considerable risk for the development of persistent or recurrent viral hepatitis after transplantation, compared with those with fulminant viral hepatitis [30, 31]; in the latter group, the virus is usually cleared by the immune system at the time of the transplantation. Patients with PSC as the cause for liver failure have the highest postoperative biliary complication rate, including the development of anastomotic stricture, which is associated with a higher rate of bacterial cholangitis and cytomegalovirus (CMV) disease [22]. It is important to remember that these patients are routinely reconstructed with CJ anastomoses, which have higher complication rates. Diabetes mellitus and invasive candidiasis have been associated with an increased rate of bacteremic infection [32, 33]. Patients with iron overload have a higher risk for fatal infections, especially those of fungal etiology [34]. In one series, hepatic iron overload assessed by the amount of stainable iron in the explanted liver was found to have a strong association with posttransplant fungal infections [35]. Transplant recipients with renal insufficiency suffer from infectious complications more frequently [13, 36]. Another study reports that high serum bilirubin at the time of transplantation is associated

with an increased rate of posttransplantation bacterial infection [11]. The hyperbilirubinemia in this setting may be reflective of the severity of the liver disease before transplantation, or it may be an indirect indicator of an undiagnosed infection at the time of transplantation. Subclinical infection before transplantation may manifest after transplantation under augmented immunosuppression. Similarly, a latent infection may reactivate after transplantation and can cause fulminant infection. This phenomenon is particularly important when caring for patients from geographic areas endemic for pathogens capable of reactivation under immunosuppression, such as *Mycobacterium tuberculosis*, endemic mycoses, and parasitic infections (e.g., Chagas disease or strongyloidiasis) [37, 38]. Also, a study by Nierenberg et al. identified pretransplant lymphopenia (<500 cells/mm^3) and CMV as independent predictors of CMV disease and non-CMV invasive infections after liver transplantation [39].

Liver transplant centers have started offering liver transplants to patients with chronic human immunodeficiency virus (HIV) infection. Most of these patients had HCV or, to a lesser degree, HBV infection as their underlying disease. Earlier experience with HIV-positive liver transplant recipients prior to the widespread use of highly active antiretroviral therapy (HAART) had shown that they had poorer outcomes, compared to those of non-HIV-infected individuals [40–42]. After the routine use of HAART, centers started offering liver transplantation to HIV-positive individuals. The largest single-center series of HIV-positive patients came from University of Pittsburgh, reporting 29 transplants performed after 1997 with 1-year survival of 89 % [43]. The indications for liver transplant included HCV (89 %), HBV (7 %), and fulminant liver failure (4 %). A prospective multicenter study was initiated in 2003 by the University of California, San Francisco, funded by the National Institutes of Health. The purpose of the study was to evaluate the safety and efficacy of liver transplantation in HIV-positive individuals [43, 44]. Eligibility for enrollment required CD4 count higher than 100 cells/mm^3 and undetectable viral load.

Most centers do not exclude from transplantation, patients who have opportunistic infections that are well controlled by HAART. Contraindications for transplantations include resistant fungal infections, progressive multifocal leukoencephalopathy (PML), and chronic cryptosporidium infection and visceral Kaposi infection [43, 44]. Consideration of the interaction between HAART and immunosuppressive agents is crucial in the management of these patients, and experience dictates how to best manage these patients. Newer antiretroviral agents, including the integrase inhibitors raltegravir, dolutegravir, and elvitegravir, have no significant interactions with tacrolimus, sirolimus, or mycophenolate, allowing HAART post liver transplantation without the need to alter traditional dosing of immunosuppressive agents. Elvitegravir has a potential interaction with cyclosporine, causing increased elvitegravir exposure. Most notorious is the interaction between protease inhibitors and CyA, tacrolimus, and sirolimus, which causes an increase in the level of these immunosuppressive agents via inhibition of the cytochrome p4503A enzymes. Most centers use steroids, CyA, or tacrolimus and mycophenolate mofetil (MMF) and do not use induction therapy agents like OKT3 and thymoglobulin [43, 44].

Of 109 HIV-infected subjects that received liver transplantation between 1999 and 2010 in a single transplant center, 37 % developed at least one episode of infection, 26 % had an episode of bacteremia, 4.6 % developed CMV infection, and only 3.6 % developed an HIV-related OI. After a median follow-up of 45.7 months, 43 patients (39.4 %) died, but infection-related mortality occurred in 9 (7 %), and in 20 (42.5 %) it was due to HCV recurrence. None of the patients died of an OI [45]. In a comparison of HIV-infected liver transplant recipients with a cohort of matched HIV-noninfected liver transplant recipients, Locke et al. found a 1.68-fold increased risk for death and a 1.70-fold increased risk for graft loss, albeit the risk in HIV-infected, HCV-noninfected recipients was similar to non-HIV-infected recipients in the modern transplant era [46].

14.3 Effect of Immunosuppression

The last decade has shown the emergence of multiple new immunosuppressive agents used in solid organ transplantation (SOT), with some used for induction therapy as lymphocyte depleting agents [47]. Agents used for immunosuppression after transplantation can be generally divided into the following categories: (a) corticosteroids; (b) cytotoxic agents, such as azathioprine and mycophenolate mofetil; (c) calcineurin inhibitors, cyclosporine, and tacrolimus; (d) TOR inhibitors, sirolimus, and everolimus; (e) monoclonal antibodies including murine anti-CD3 monoclonal immunoglobulin OKT3, the anti-CD25 monoclonal antibodies basiliximab and daclizumab, and alemtuzumab a relatively new monoclonal antibody used as a lymphocytic depleting agent for induction and for rejection therapy; and (f) polyclonal antilymphocyte antibodies including rabbit polyclonal antithymocyte gamma globulin (Thymoglobulin) and equine polyclonal antithymocyte gamma globulin (equine antithymocyte globulin [ATG]) [47, 48]. The pretransplantation use of corticosteroids is associated with an increased risk of systemic *Candida* infection in liver transplant recipients [16]. With the current practice of maintaining patients on low-dose corticosteroids, except in the case of acute rejection, steroid-related opportunistic infections are not as common as they used to be when a higher dose was routinely used.

Calcineurin inhibitors are now the mainstay of the immunosuppressive therapy in solid organ transplant recipients. Tacrolimus is a much more potent agent than cyclosporine, and it is associated with fewer rejection episodes and prolonged graft survival. Studies comparing the two agents in liver transplantation show that patients receiving tacrolimus have a tendency to contract fewer infections [3, 49]. This

may be related to the reduced requirement for corticosteroids and antilymphocyte agents in the tacrolimus group. The use of MMF was not found to be associated with increased risk of infection [50]. Sirolimus was introduced to immunosuppressive protocols because of its lack of nephrotoxicity but has been associated with increased rates of infection [51]. Antilymphocyte monoclonal murine antibody OKT3 has been associated with increased CMV infection [52, 53]. Patients who have been treated with OKT3 also have an increased rate of symptomatic herpes simplex virus (HSV) reactivation, *Pneumocystis carinii* infection, and posttransplant lymphoproliferative disease [1, 52, 54]. Current use of OKT3 is rare. Thymoglobulin has been used with increasing popularity by centers wishing to avoid steroids. Alemtuzumab has been found to be associated with increased rate of opportunistic infection when given for treatment of rejection but not when given for induction therapy [55].

14.4 Infecting Microorganisms: Epidemiology, Risk Factors, and Prevention

Many factors influence the incidence of posttransplantation infections, including surgical techniques, the degree of immunosuppression, and donor and recipient factors as already discussed. Whether a patient develops a certain type of infection at a given time is also highly dependent on the time elapsed since transplantation, as Figure 14-5 illustrates. Table 14-1 summarizes risk factors for infections by different classes of pathogens. Table 14-2 lists commonly used prophylactic agents against several important infective agents that affect liver transplant recipients.

14.5 Bacterial Pathogens

Most bacterial infections in liver transplant recipients occur within the first month after transplantation [11]. Table 14-3 shows the breakdown by type of bacterial infections as seen in several studies from the 1980s. Intra-abdominal infection (IAI), including peritonitis, intra-abdominal and hepatic abscess, and cholangitis, was the most common type of infection. Aerobic gram-negative bacilli (e.g., *Escherichia coli*, *Enterobacter*, *Pseudomonas*) were most frequently implicated, followed by *Enterococcus* and *Staphylococcus*. Centers using selective bowel decontamination (SBD) saw significant decreases in gram-negative pathogens but increases in aerobic gram-positive organisms in both adult and pediatric transplant population [2, 56, 57]. Anaerobes other than *Clostridium difficile* were recovered in less than 10% of the infections [11]. Although infections by gram-negative bacilli still confer substantial morbidity and mortality in this population, the worsening antibiotic resistance

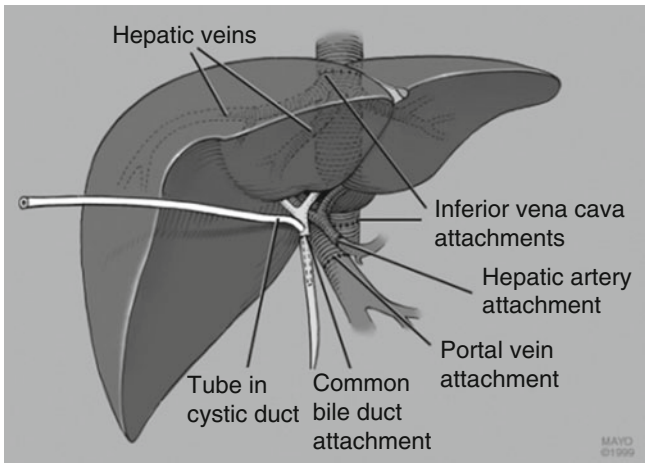

FIGURE 14-5. Incidence in episodes of infections per patient per year and time of occurrence after transplantation when severe infections occurred (Reproduced from Kusne S, Dummer JS, Singh N, et al. Infections after liver transplantation: an analysis of 101 consecutive cases. Medicine (Baltimore). 1988;67:132–143, with permission).

patterns in nosocomial gram-positive pathogens, such as MRSA and VRE, are becoming increasingly problematic.

A recently published meta-analysis by Ziakas et al. [58] reported an 8.5% MRSA pretransplant colonization rate in solid organ transplant, as well as a high VRE colonization rate of 11.9% pretransplant and 16.2% posttransplant. MRSA colonization was associated with a 6–11-fold increase in MRSA infection. A bundled intervention study by Schweizer et al. [59] consisting of *Staphylococcus aureus* preoperative screening, decolonization with mupirocin intranasally, and chlorhexidine daily washes and targeted MRSA preoperative prophylaxis in orthopedic and cardiac surgeries have shown a significant reduction in postoperative infections [59].

MRSA nasal colonization is correlated with the increased MRSA infection in liver transplant units [60–62]. Usage of nasal mupirocin only temporarily eliminated nasal colonization and does not prevent MRSA infection. Strict infection control measures were effective in decreasing significantly both *S. aureus* colonization and infection [63]. Bakir [64] noted a VRE colonization rate as high as 55% among liver transplant recipients, two thirds of whom acquired the organism after transplantation, thus suggesting cross-contamination in the ICU. Singh [65] also reported a high rate of infection by resistant bacteria among liver transplant recipients. MRSA was implicated in 91% of all infections by *Staphylococcus* species, and VRE in 50% of all enterococcal infections. A large proportion of gram-negative infections were from multiresistant *Pseudomonas* species (47%) and *Enterobacteriaceae* (60%) [65]. Invasive infection by VRE, in particular, as a part of IAI or catheter-related bacteremia is commonplace in many liver transplant ICUs. Linden compared in 1996 the outcome of consecutive liver transplant recipients with *Enterococcus faecium* bacteremia resistant

TABLE 14-1. Major risk factors for various infections after liver transplantation

Type of infection	Surgery-related factors	Host-related factors
Bacterial	Prolonged surgical time	Diabetes mellitus
	Retransplantation	Hyperbilirubinemia
	High transfusion volume	Recipient with primary sclerosing cholangitis
	Choledochojejunostomy	Previous granulomatous disease
	Hepatic artery thrombosis	
Viral	Not clearly defined	Use of antilymphocytic antibodies and steroids
		Seropositive donor and seronegative recipient (CMV, EBV)
		Acute fulminant hepatitis as indication for transplant
		Donor positive for hepatitis core antibody (HBV)
Fungal	Prolonged surgical time	Diabetes mellitus
	Retransplantation	Iron overload of recipient
	High transfusion volume	Use of corticosteroids
	Poor function of allograft	Use of antilymphocytic antibodies
		Renal insufficiency
		CMV infection
		Fungal colonization
		Previous endemic fungal infection
		Treatment with multiple antibiotics

Abbreviations: *CMV* cytomegalovirus, *EBV* Epstein-Barr virus, *HBV* hepatitis B virus.

TABLE 14-2. Prophylactic regimen used for various pathogens after liver transplantation

Pathogen	Prophylaxis
Bacterial	
Routine postoperative infection	Perioperative IV antibiotics for GI pathogens for 48 h, selective bowel decontamination with PO polymyxin E and gentamicin in some centers
Streptococcus pneumoniae	Pneumococcal vaccine pretransplant and an additional revaccination after 5 years
Mycobacterial (TB)	Isoniazid for 9 months
Fungal	
Candida	PO nystatin, PO fluconazole, short-course IV liposomal amphoB
Aspergillus	Surveillance cultures and possibly galactomannan antigen
Pneumocystis	TMP/SMX
Protozoan	
Toxoplasma	TMP/SMX, pyrimethamine
Viral	
CMV	IV ganciclovir, oral ganciclovir, oral valganciclovir, CMV IVIG
HSV	PO acyclovir
HBV	HBIG, lamivudine
HCV	No clear recommendation
Influenza	Yearly influenza vaccine and antiviral agents (zanamivir, oseltamivir) in an outbreak setting

and susceptible to vancomycin [66]. VRE bacteremia was associated with refractory infection with serious morbidity and higher mortality [66]. More recent investigation by Pelz [67] has not found differences between vancomycin-sensitive or vancomycin-resistant enterococcal infection in terms of cost of ICU care and mortality [67]. This may be most likely related to the use of agents with anti-VRE activity, such as linezolid and Synercid (quinupristin/dalfopristin) and daptomycin. The emergence of VRE resistant to linezolid has already been reported [68]. Strict enforcement of contact precautions using barrier methods and scrupulous handwashing cannot be overemphasized in this vulnerable population.

While resistant gram-positive bacterial infections dominated the medical literature in the past two decades, both, a resurgence of multidrug-resistant GNR (MDR-GNR) and the scarcity of newer antimicrobials with activity against MDR-GNR, have made the treatment of these infections more complex with poor outcome. A report from Kalpoe et al. [69], in a cohort of 175 consecutive liver transplant recipients from January 2005 to October 2006, illustrates the change. Within

Table 14-3. Rates and locations of bacterial infection after liver transplantation at different centers

Authors (ref.)	Study period	Number of patients evaluated	Patients with major bacterial infection (%)	Perioperative prophylaxis	Total number of bacterial infection	Number of episodes of bacteremia	Number of episodes of infection by type	
							Type	Number
Colonna [64]	1984–1985	35	NR[a]	PO erythromycin, neomycin pre-op., IV ampicillin, and gentamicin post-op. for 5 days	37	11	IAI	10
							Wound	8
							Pneumonia	4
							Line	5
Kusne et al. [1]	1984–1985	101	54	IV ampicillin, cefotaxime×5 days post-op	80	33	IAI	32
							Wound	10
							Pneumonia	15
							Line	2
Paya et al. [2]	1985–1987	53	36	PO polymyxin E, nystatin, gentamicin pre-op., and then 21 days post-op. IV cefotaxime, tobramycin×2 days	27	16	IAI	13
							Wound	–
							Pneumonia	4
							Line	2
George et al. [11]	1985–1987	79	69[b]	IV cefoxitin×1–2 days post-op.	115	31	IAI	35
							Wound	19
							Pneumonia	11
							Line	4

Abbreviations: *IAI* intra-abdominal infection, including abscess, cholangitis, and peritonitis.
[a]Percentage of all infection (including fungal and viral) was 66%; separate bacterial infection rate not specified.
[b]All bacterial infection, both major and minor; severity of infection not specified.

1 year of liver transplantation (LT), 35% of patients ($n=61$) have developed 91 bacterial infections. Sixty-one episodes (67%) of bacteremia occurred in 34 patients, 45 episodes of peritonitis, and 35 episodes of IAI. Polymicrobial infections were seen in 31% of the episodes. Gram-negative bacteria caused 69% of the episodes. Enterococcus was the most commonly isolated in 43 of the episodes, followed by *Klebsiella* species in 37%. Multidrug-resistant *Klebsiella* (both carbapenem-resistant and carbapenem-susceptible extended spectrum beta-lactamase producer) was the most commonly isolated strains of *Klebsiella* species representing 28/34 isolates (82%). One-year survival was lower in patients with a bacterial infection (67% vs. 90%). Seventy-one percent of the 14 patients with CrKp infections died a median of 15.5 days from infection diagnosis [69]. Treatment of CrKp infections is not standardized yet, and only few antibiotics retain activity. Options include monotherapy or combination therapy with tigecycline, colistin-polymyxin B, and ceftazidime-avibactam. Others have used meropenem in combination with tigecycline or colistin.

Multidrug-resistant organisms, named ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.), are becoming more frequently isolated in posttransplant infections. A retrospective review of episodes of bacteremia in solid organ transplant recipients from 2007 to 2012 showed that 19.6% of bacteremias were due to ESKAPE organisms. The median time to bacteremia was shorter that other organisms (59 vs. 331 days). Risk factors included a history of prior transplantation, septic shock, and prior antibiotic treatment. More importantly, 41% of subjects received initial inappropriate antimicrobial treatment, and the case fatality rate was higher (35.2% vs. 14.4% [70]).

Carbapenem-resistant *Klebsiella pneumoniae* (CrKp) has become an increasingly challenging organism to treat. CrKp infections in liver transplant recipients are associated with increased mortality (50% survival at 1 year vs. 93% survival if no *Klebsiella* infection). Predictors of CrKp infection included MELD score at the time of transplantation, the presence of hepatocellular carcinoma, Roux-en-Y choledochojejunostomy, and formation of bile leak [71].

The causative organisms in postoperative bacterial infections usually originate from the patient's endogenous flora. The commensal flora of a transplant recipient frequently changes in the postoperative period, with more extensive colonization with nosocomial pathogens occurring with longer hospital stays after transplantation [72]. Breaches in infection control practices propagate the patient-to-patient spread of such pathogens through contact. Infections from purely exogenous sources, such as *Pseudomonas* species from contaminated bronchoscopes [73] or *Legionella* species from contaminated water supplies [74], have been reported, but these are relatively uncommon. Another major bacterial agent that has been implicated in postoperative infection is *C. difficile*, which has been reported in 3–6% of liver transplant recipients [1, 11]. Prolonged ICU stay and the frequent use of broad-spectrum antibiotics with antian-

aerobic activities, which have long been identified as significant risk factors for *C. difficile*-related diarrhea, are common in this group of patients [75]. Although *C. difficile* colitis is readily treatable, in this population, it may have a fulminant presentation that leads to toxic megacolon, the need for a colectomy, and death. Between 2000 and 2003, a new strain of *C. difficile* with increased virulence and resistance emerged and caused outbreaks in six states in the United States including Georgia, Illinois, Maine, New Jersey, Oregon, and Pennsylvania, leading to increased morbidity and mortality [76]. A large retrospective review from the 2009 Nationwide Inpatient Sample database extracted information on 49,198 cases of SOT. Overall, 2.7 % of cases acquired *Clostridium difficile* infection, with liver transplant recipients having the highest incidence of 3.8 %. *Clostridium difficile* infection in the SOT group was associated with approximately 2.5 times higher in-hospital mortality, 10 days longer hospital stay, and $70,000 in additional charges in patients with SOT [77]. Current treatment options for *Clostridium difficile* include metronidazole, oral vancomycin, and fidaxomicin. Close surveillance of patients at risk, the prompt discontinuation of unnecessary antibiotics, and diligent infection control practices are vital in reducing the rate of infection by *C. difficile*. Overall, the mortality rate directly attributable to bacterial infections in liver transplant recipients ranges from 3.8 to 13.3 % [1–3, 11, 17].

In 2004, a consensus document was published that summarized the surgical infection prevention guidelines regarding bacterial prophylaxis [78]. Antibiotics chosen for prophylaxis are given within 60 min before the first incision and if surgery continues two half-lives after the first dose, a second dose is administered. It is usually not recommended to continue bacterial prophylaxis more than 24 h after the operation [78]. The main goal of prophylaxis is to prevent wound infection by using agents that are active against expected organisms in the operation [78]. The liver transplantation wound is considered clean contaminated unless spillage from the GI tract occurs.

Most liver transplant centers administer routine surgical prophylaxis with broad-spectrum antibiotics for 48 h after the surgery [11]. Some agents include ampicillin and cefotaxime, ceftriaxone, ampicillin and gentamicin, ampicillin and sulbactam, and cefoxitin. These antibiotics are specifically chosen for their efficacy against enteric gram-negative bacilli and *Enterococcus* organisms. Researchers from Mayo Clinic, Rochester [2], had noted a disproportionately low infection rate caused by gram-negative organisms in their series and hypothesized that the routine use of "selective bowel decontamination" targeted against gram-negative organisms in their institution selected for gram-positive pathogens. Several studies have examined the role of SBD in the prevention of infection in liver transplant recipients without reaching conclusive results regarding its benefits [79–83]. The rationale for SBD is to eradicate or to minimize GI colonization by gram-negative pathogens and yeasts. Earlier studies did suggest some benefits in reducing gram-negative infections, particularly pneumonia. However, two recent randomized, controlled trials using oral polymyxin E, gentamicin, and nystatin vs. a placebo [80] and nystatin alone [83] did not show any improvement in overall bacterial or fungal infection. One study showed a trend toward reduced infection in "key sites" (i.e., abdomen, bloodstream, surgical wound, and lungs) when the prophylaxis was given for longer than 3 days before transplantation [80]. The lack of benefits and the concerns regarding the selection of resistant gram-positive pathogens have curtailed the widespread use of SBD in transplant centers.

14.5.1 Fungal Pathogens

Liver transplant recipients encounter fungal infections more often than recipients of other solid organs. The reported incidence of IFI ranges from 4 to 42 %, with a case fatality rate of 24–69 % [1, 2, 12–16]. Most fungal infections occur within the first month of the transplantation [13].

14.5.1.1 Risk Factors

Risk factors for IFI include elevated creatinine levels, prolonged operation time, poor function of the allograft, high transfusion requirement, retransplantation, and CMV infection [1, 13–16, 36, 84–86]. Some studies show an association between IFI and the preoperative use of steroids, the use of multiple antibiotic therapy, and vascular complications [14, 16]. Colonization with *Candida* species was shown to be a significant predictor for invasive candidiasis, although the risk of infection did not rise with an increasing number of sites of colonization [13]. Other risk factors for candidiasis include hyperglycemia that requires insulin and exposure to multiple antibiotics [32]. At least one study [84] showed that prophylactic intravenous (IV) ganciclovir was an independent risk factor for invasive candidiasis. The reason for this finding was unclear; the authors hypothesized that ganciclovir may reduce neutrophil or macrophage function. The need for an intravascular catheter for ganciclovir administration may also have contributed by becoming a portal of entry for *Candida* organisms. Because the number of patients with invasive *Aspergillus* infection is small in most series, finding specific risk factors for this infection is more difficult. In a large series of *Aspergillus* infection in liver transplant recipients, Kusne et al. [36] identified cumulative surgical time, laparotomies excluding those done for transplantation, creatinine level at the time of the diagnosis, and OKT3 monoclonal antibody administration as risk factors for invasive aspergillosis in univariate analysis, but only creatinine level and OKT3 were shown to be independent risk factors in the multivariate analysis. Immunosuppression plays an important role in the risk for cryptococcosis. Use of systemic corticosteroids at the time of the diagnosis has specifically been shown to have an association with an increased rate of cryptococcal infection [85–88].

14.5.2 Candidiasis

The most commonly implicated fungal pathogen is *Candida* species, which account for approximately 80 % of all IFIs [14, 72, 84]. The rate of GI colonization with *Candida* is about 30–60 % in healthy subjects, but it approximates 100 % in liver transplant recipients [89, 90]. A high rate of colonization before transplantation, the subsequent breach of mucosal integrity through surgery, and the frequent postoperative use of broad-spectrum antibiotics in this population all contribute to predisposing these patients to IFIs. Surgical wounds and the abdominal cavity are the most common sites of infection by *Candida* species, followed by bloodstream infection associated with the central venous catheter, esophagitis, and disseminated disease. Recently, the incidence of infection by non-albicans species, such as *Candida glabrata* or *Candida krusei*, has been rising [91]. This is related to routine administration of antifungal prophylaxis, specifically fluconazole, and is associated with higher mortality [92]. An infection with non-albicans candidiasis usually requires therapy with amphotericin B (amphoB). The introduction of echinocandins (caspofungin, anidulafungin, and micafungin) has added new therapeutic armamentarium against invasive *Candida* infections. Some advantages of echinocandins are their fungicidal activity, in vitro increased activity against *Candida*-forming biofilm, and postantifungal effects. Disadvantages include the lack of therapeutic concentrations in CSF, urine, and aqueous/vitreous humor and the need for intravenous administration. There is improved clinical success using an echinocandin for treatment of *Candida glabrata* fungemia. On the other side, *Candida parapsilosis* has a higher MIC, and there is no outcome difference between an echinocandin and azole treatment [93]. The use of amphoB is problematic, given its nephrotoxicity and the high rate of pre-existing renal insufficiency in liver transplant recipients, as well as the concurrent use of other potentially nephrotoxic medications, such as cyclosporine and tacrolimus. In 2002, a double-blind trial demonstrated that caspofungin, an echinocandin, was as effective as amphoB for treatment of invasive candidiasis and at the same time has fewer adverse effects compared to amphoB [94]; cases in the two arms were equivalent in severity of illness and rate of neutropenia. According to the Infectious Disease Society of America (IDSA) Clinical Guidelines [95], the initial coverage of candidemia and disseminated candidiasis include caspofungin or fluconazole or amphotericin B.

14.5.3 Aspergillosis

Aspergillus species are the second most frequent cause of IFIs in this population, and they are associated with an extremely high mortality rate that ranges from 75 to 100 % [1, 13–16, 84]. A large case series reported that 32 of 2180 patients (1.5 %) were diagnosed with aspergillosis over the period of 10 years [36]. In 41 % of the cases, the diagnosis was made postmortem. Twenty-seven (84 %) had pulmonary involvement, two had intra-abdominal disease, and one had central nervous system (CNS) infection. The diagnosis was made in 90 % of the patients within the first 50 days of the transplantation. Although the authors identified a group of patients with a positive culture for *Aspergillus* species who did not have evidence of tissue invasion, most patients (68 %) whose culture was positive did have invasive aspergillosis. Therefore, the authors concluded that the isolation of *Aspergillus* species from the respiratory tract of transplant recipients could not be ignored as simple colonization, as it often is in patients with normal immune systems. With the advent of effective new antifungal agents against *Aspergillus* species, such as liposomal amphoB and voriconazole, the mortality rate of invasive *Aspergillus* infection has improved in liver transplant recipients. A small case series [96] described several liver transplant recipients with invasive aspergillosis who were treated successfully with a prolonged course of amphotericin lipid complex, followed by oral itraconazole. Linden et al. compared the outcome in a retrospective study of liver transplant recipients with invasive aspergillosis treated with amphoB lipid complex to those treated with conventional amphoB [97]. The 60-day survival was statistically higher in the lipid formulation-treated patients [97]. In a randomized trial, Herbrecht et al. compared the treatment of invasive aspergillosis with voriconazole vs. deoxycholate amphoB in allogeneic hematopoietic cell transplantation and acute leukemia [98]. Better response and better survival was observed in the voriconazole arm [98]. In the IDSA 2008 Clinical Practice Guidelines, voriconazole is recommended for primary treatment of invasive aspergillosis [99]. Alternative treatments include liposomal amphotericin B, caspofungin, micafungin, posaconazole, and itraconazole [99].

Some centers use combination antifungals to treat refractory cases of invasive aspergillosis. This approach is controversial with conflicting in vitro and in vivo reports. A recent in vitro study suggested that the combination of amphotericin B and voriconazole was more effective than monotherapy [100]. A randomized trial, combining anidulafungin and voriconazole for treatment of invasive aspergillosis in patients with hematological malignancies and stem cell transplantation, suggested a reduced mortality in the combination therapy arm (19.3 % vs. 27.5 %) [101]. Resistance of aspergillus to azoles has become more frequent. Fuhren et al. [102] reported on 21 of 105 *Aspergillus fumigatus* resistant in vitro to at least one azole (voriconazole, posaconazole, or itraconazole), and 16.2 % (17/105) were resistant (MIC>2 mg/L) to voriconazole in high-risk patients (ICU and hematology ward). The authors suggested to check for resistance prior to initiation of treatment. A recently approved azole, isavuconazole, has broad activity against mold, including *Aspergillus fumigatus* and *A. flavus*, as well as activity against some *Mucorales* and dimorphic fungi. In a recent randomized, double-blind clinical trial for treatment of invasive

aspergillus infection, isavuconazole was noninferior to voriconazole; there was no difference in day 42 all-cause mortality and overall success (complete and partial response) [103].

14.5.4 Cryptococcosis

Contrary to infections by *Candida* or *Aspergillus* species, infection by *Cryptococcus neoformans* tends to occur several months after transplantation. In a series of ten cases of *Cryptococcal meningitis* in liver transplant recipients, Jabbour et al. [104] reported the median time elapsed since transplant to the diagnosis as 3.5 months (range of 2–36 months). A large review by Husain et al. [105] that researched cryptococcosis in all types of solid organ transplant recipients suggested an even later onset of infection. The median time of onset was 1.6 years after transplantation in that study, with 59 % of the cases occurring more than 12 months after transplantation. The infection did occur earlier in liver and lung transplant recipients, compared with that of heart and kidney transplant recipients, possibly as a result of the more aggressive early immunosuppressive regimen used in these groups of patients. The rate of cryptococcosis was not as high in liver transplant recipients as it was in those receiving heart transplants. The most commonly involved site was the CNS (72 %), followed by the lungs (25 %), skin, soft tissue, joints, and bones, with 24 % of the patients having more than one site of infection. The overall mortality in the series was 42 %. The incidence of the infection also differs according to geographic areas. For example, cryptococcosis is much more common in the eastern United States than it is in the western part of the country. Men are more frequently affected than women [106]. Mortality was associated with organ failure, liver failure in liver transplant recipients [107], and renal failure in all transplant organ recipients [86].

Cryptococcal meningitis or other severe cryptococcal disease infection is usually treated with induction treatment with liposomal AmB 3–4 mg/kg daily or ABLC 5 mg/kg per day together with flucytosine 100 mg/kg daily for 2 weeks [108]. This is followed by oral fluconazole 400–800 mg/day for 8 weeks (consolidation therapy) and then fluconazole 200–400 mg daily for 6–12 months (maintenance therapy). Induction therapy can also be given with daily 4–6 weeks of liposomal AmB 6 mg/kg or ABLC 5 mg/kg or AmBd 0.7 mg/kg [108].

Between 2004 and 2010, *Cryptococcus gattii* emerged as an important pathogen in the Pacific Northwest [109]. This species had appeared first in animals and humans in Vancouver Island and in 2004 in mainland British Columbia, and only 38 % of the patients were immunosuppressed including solid organ recipients [109]. Forrest et al. described 11 solid organ transplant recipients in the Oregon outbreak [110]. Solid organ transplant recipients had higher rate of dissemination and higher mortality compared to nonimmunosuppressed host [110].

14.5.5 Prevention Strategies

Two strategies to prevent IFIs in liver transplant recipients, universal prophylaxis and targeted prophylaxis, have been used, but a consensus has not been achieved regarding the optimal regimen for prevention. Oral nystatin administered as part of SBD has been shown to reduce oropharyngeal and rectal colonization with *Candida* species, but no evidence appears to indicate that it reduces the rate of IFI [79]. Nonetheless, several centers routinely use it because it is an inexpensive and benign therapy. Oral fluconazole has been examined in two randomized, controlled studies for its potential for the prevention of IFI caused by *Candida* species [111, 112]. One study compared a low-dose fluconazole, 100 mg daily, with oral nystatin for a month after transplantation. The fluconazole group showed a trend toward lower IFI rates, without significant toxicity or interference with cyclosporine among the study group patients [95]. A larger study by Winston et al. [112] used a fluconazole dose of 400 mg daily. The benefits of fluconazole prophylaxis were clearer in this study, with a reduced rate of IFI (9 % vs. 43 % in the control group) and fewer deaths secondary to fungal infection in the fluconazole group. The tradeoff was a higher cyclosporine level in the study group, which translated into increased neurotoxicity. However, the all-cause mortality was similar in the two groups. Interestingly, none of the studies showed an increased rate of infection by fluconazole-resistant *Candida* species, such as *C. glabrata* or *C. krusei*. An earlier study by Tollemar et al. [113] looked at the protective effect of low-dose liposomal amphoB (AmBisome), 1 mg/kg daily, compared with a placebo; both were administered during the first 5 days after the transplantation. None of the 40 patients in the AmBisome group contracted IFI, whereas 6 of 37 patients in the control group developed IFI (5 cases of invasive *Candida* infections and 1 case of *Aspergillus* pneumonia). The problem with this strategy lies mainly with the prohibitive cost of AmBisome, although the low dosage and short duration of prophylaxis help mitigate the issue.

The IDSA guideline is to use prophylactic antifungal therapy during the early postoperative period in high-risk liver transplant recipients [95]. According to some reports, universal antifungal prophylaxis, specifically fluconazole, has been associated with increased rate of IFI secondary to non-albicans isolates [92, 114]. Also, the azole antifungals interfere with the metabolism of immunosuppressive agents, leading to increased creatinine secondary to accumulation of tacrolimus and cyclosporine. Compared to universal prophylaxis, targeted prophylaxis is defined as the selective targeting of only patients at high risk for IFI, such as patients with renal insufficiency, long operative time, and significant intra-abdominal bleeds. The following are two examples of usage of this strategy. Fortun et al. [115] administered a lipid formulation (cumulative dose of 1–1.5 g of AmBisome or Abelcet) to high-risk liver transplant recipients and saw a decrease of IFIs (17–6 %) and aspergillosis

(10–4 %), compared to a historical group [115]. Hellinger et al., at Mayo Clinic, Florida [116], stratified patients during targeted prophylaxis intervention into risk-groups and administered Abelcet 5 mg/kg to those at high risk for development of IFI. During the intervention with targeted prophylaxis, there was only 1 % invasive mold infection compared to 5 % before institution of targeted prophylaxis [116].

14.5.6 Viral Pathogens

14.5.6.1 Cytomegalovirus Infection

CMV is one of the most common infections, and it is a major source of morbidity and mortality in liver transplant recipients. Studies done prior to the widespread use of CMV prophylaxis or preemptive therapy showed that 60 % of all liver transplant recipients developed CMV infection, with symptomatic infections appearing in 32–56 % [52, 117].

Along with common presentations of symptomatic CMV disease, such as viral syndrome, CMV gastroduodenitis, colitis, and pneumonitis, CMV disease in liver transplant recipients often presents as hepatitis, an entity that is rarely observed in recipients of other organs. In a study of 50 liver transplant recipients with CMV infection, CMV hepatitis was observed in 13 (26 %) [117]. In that study, the most important risk factor for CMV infection for both symptomatic and non-symptomatic cases was mismatched donor and recipient serostatus, in which CMV-seronegative recipients received CMV-seropositive organs [53, 117, 118]. The use of OKT3 anti-CD3 monoclonal antibody was a significant risk factor for symptomatic CMV infection, especially in those who were CMV seropositive before transplantation [119]. More recently, the use of polyclonal antithymocyte antibodies (rabbit, Thymoglobulin, and equine, ATG) was associated with CMV reactivation. A prolonged prothrombin time and the diagnosis of acute fulminant hepatitis as the underlying liver disease have also been associated with an increased rate of CMV infection [53]. Moreover, studies indicate that CMV infection alone is an independent risk factor for IFIs [13, 85], thus strengthening prior claims that the virus itself is an immune modulator and has depressive effects on cellular immune function [120, 121].

Because of the high prevalence of, and morbidity associated with CMV infection, considerable efforts have been made to determine the best strategy for preventing CMV disease in this population. Acyclovir and ganciclovir have been studied as both universal prophylaxis and preemptive therapy. Although at least one trial has attested to the efficacy of acyclovir in the prevention of CMV infection in liver transplant recipients [122], subsequent studies have shown acyclovir to be inferior to ganciclovir in both prophylactic and preemptive strategies [123–127]. Subsequently, IV ganciclovir has been established as the mainstay of CMV prophylaxis and treatment. When IV ganciclovir is used as an agent of universal prophylaxis for 100 days after transplantation, it dramatically reduced the rate of CMV infection in both seropositive and seronegative liver transplant recipients, compared to that seen with acyclovir use [123]. The protective effect of IV ganciclovir was preserved but blunted when it was administered for only 2 weeks, followed by oral acyclovir [125]. IV ganciclovir was also effective when it was used in preemptive therapy guided by results of the surveillance CMV pp65 antigenemia test [126, 128].

The oral form of ganciclovir has the advantage of ease of administration over that of the IV formulation. Despite several studies that demonstrate the efficacy of oral ganciclovir in both prophylactic and preemptive strategies [124, 126, 129], many clinicians have expressed reservations about the routine use of this agent due to its suboptimal bioavailability and concerns about fostering resistance. Oral valganciclovir (Valcyte) is the L-valyl ester of ganciclovir and is rapidly converted into ganciclovir and has a good bioavailability [130]. In a randomized prospective trial of 900 mg once a day of valganciclovir vs. 1000 mg three times a day of oral ganciclovir for 100 days, in high-risk solid organ recipients, CMV disease was observed in 12.1 % and 15.2 % in the valganciclovir and the oral ganciclovir arms, respectively, within 6 months from transplantation [131]. But in a subgroup analysis, the rate of CMV disease in liver transplant recipients was higher in the valganciclovir arm compared to the oral ganciclovir arm (19 % and 12 %, respectively) [131]. Valganciclovir has a black box warning from the FDA for the use of prophylaxis in liver transplantation. Despite this warning, most transplant centers use valganciclovir for prophylaxis and preemptive therapy in liver transplant recipients. Although in the seropositive-recipient setting both prophylaxis and preemptive therapies are practiced, in the high-risk setting, most clinicians would use 3 months of valganciclovir prophylaxis [132]. In recent years, more attention has been given to delayed and late CMV infections. With the increase in antiviral prophylaxis use, especially in high-risk transplant recipients, more patients present with CMV infection after finishing their course of prophylaxis. In the Limaye et al. series, the median onset of CMV disease was 4.5 months after transplantation and occurred mostly in high-risk individuals and was associated with increased risk of mortality [133]. Occasionally CMV disease can be encountered more than a year after transplantation (late CMV). This occurs usually in seropositive transplant recipients, most likely secondary to reactivation [134].

Newer therapeutic agents for treatment and prevention of CMV disease are in development. Brincidofovir (CMX001), a new broad anti-DNA virus agent, has shown in a recent study a significant reduction in the incidence of CMV disease in recipients of SCT (10 % vs. 37 % with placebo) [135]. There was no increased myelosuppression and no nephrotoxicity, and none of the recipients of CMX001 at a dose of

100 mg twice a week developed CMV disease nor viremia above 1000 copies [135]. No published data on the use of CMX001 in liver transplant population is available yet.

Letermovir, a novel terminase inhibitor, is in development for the prophylaxis and treatment of CMV. In a phase 2 randomized, controlled, multicenter, open-label trial, two doses of letermovir (40 mg BID or 80 mg daily) were compared with standard of care in adult kidney transplant recipients with active CMV replication. Despite a small sample, and a short treatment of 14 days, no patients in the three study arms developed CMV disease, and no severe adverse events were reported [136]. A phase 2 study using letermovir for CMV prophylaxis in SCT recipients showed a dose-dependent reduction in the incidence of CMV prophylaxis failure (48 % at a daily dose of 60 mg, 32 % at a dose of 120 mg, and 29 % at a dose of 240 mg vs. 64 % with placebo) with no significant hematological toxicity or nephrotoxicity [137].

Leflunomide, an antirheumatic medication with antiviral and immunosuppressive properties, has been used for the past 10 years to treat CMV. In a retrospective series from the University of Chicago, five kidney transplant recipients with ganciclovir-resistant CMV were treated with leflunomide as "consolidation" therapy after clearance of viremia with IV foscarnet without any relapses of CMV viremia while on leflunomide treatment [138].

Maribavir, a promising oral agent with potent in vitro activity against CMV, was evaluated as a prophylactic agent in liver transplant recipients at a dose of 100 mg twice a day and was compared to oral ganciclovir 1000 mg three times a day. Maribavir was found to be inferior to ganciclovir at the study dose, with confirmed CMV disease or viremia in 20 % of ganciclovir recipients vs. 60 % of maribavir recipients at day 100 and 53 % vs. 72 % at 6 months [139].

14.5.6.2 Herpes Simplex Virus

HSV infection appears within 2–3 weeks of transplantation in recipients who do not receive antiviral prophylaxis [140, 141]. The disease occurs mainly through viral reactivation in seropositive individuals, although primary infection that may cause fulminant disease is possible. Orolabial mucocutaneous manifestation is the most common presentation, followed by anorectal disease and HSV esophagitis. Disseminated disease, which usually causes hepatitis, is uncommon (0.3 %) but devastating when seen. In a series from Pittsburgh [140], 42 % of all liver transplant recipients with HSV hepatitis developed widespread disease and disseminated intravascular coagulopathy (DIC). All patients who developed DIC died [121]. Several studies have shown that prophylaxis against HSV infection with acyclovir is effective in renal and liver transplant recipients [140, 142]; many centers therefore include this as a part of the routine postoperative regimen. Valganciclovir which is used commonly as a CMV antiviral prophylaxis in solid organ transplant recipients is also an effective agent for HSV prophylaxis [143].

14.5.7 Adenovirus

Adenovirus, which is a nonenveloped DNA virus, may affect both normal and immune-compromised hosts including transplant recipients, especially pediatric but occasionally also adult [144]. The clinical presentation may be confused with CMV infection because inclusion bodies can be seen with histology and unless special stains for adenovirus are performed clinicians may be misled by initial clinical impressions.

Similar to CMV, adenovirus infection may be asymptomatic or symptomatic causing invasive disease. In pediatric liver transplant recipients, hepatitis is the most common invasive disease [145]. Other infections include hemorrhagic cystitis, gastroenteritis, pneumonitis, and disseminated infection. In one series, 49 (10 %) of 484 children on cyclosporine and steroids developed adenovirus infection at a median of 25.5 days after liver transplantation and 20 (41 %) developed symptomatic infection and nine died [146]. There was a statistical trend of OKT3 use in patients with invasive disease compared to noninvasive infection. Nine (64 %) of 14 children who developed adenovirus hepatitis were type 5 and the others were types 1 and 2 [146]. Patients with adenovirus hepatitis were systemically ill with high temperature and their transaminase levels were elevated [147]. Liver biopsy demonstrated parenchymal punched-out lesions with some necrosis and a scant number of intranuclear inclusions [147]. Extensive necrosis can be seen in fulminant adenovirus hepatitis [147]. Cidofovir has in vitro activity against adenovirus and has been used with mixed results. Successful outcomes were reported in cases where immunosuppression was reduced, and treatment was instituted relatively early in the course of the infection [147, 148]. Most recently, quantitative PCR has been used by some investigators to follow patients' clinical progress with adenovirus infection as high viral load correlated with severe symptomatic infection [147, 148].

14.5.7.1 Recurrent HBV

HBV and HCV are two of the most common indications for liver transplantation. Although HBV infection has been declining as an indication for liver transplantation, HCV infection, which often goes unrecognized for years, is rising; it currently is the most common cause for end-stage liver disease requiring liver transplant in the United States [88]. Patients with HBV or HCV who undergo transplantation have a considerable risk of recurrent infection and may develop cirrhosis at an accelerated rate.

HBV recurrence occurs in more than 80 % of liver transplant recipients who have HBV before transplantation [149, 150]. Active HBV viral replication and the presence of the HBe antigen before transplantation have been associated with an increased risk of recurrent hepatitis secondary to HBV, whereas those with fulminant hepatitis as the indication for transplant or superinfection by the delta virus have been shown to have lower rate of recurrent infection [30].

Hepatitis B serology of the donor may determine whether the recipients with HBV-related cirrhosis develop viral hepatitis after transplantation. According to one study, HBV-positive transplant recipients who received organs from donors with positive anti-HB core antibody show 2.5 times the risk of recurrent HBV hepatitis after transplantation compared to those who received organs from donors without HBV markers [151]. However, the study did not show any difference in graft or patient survival. De novo hepatitis B infection in HBV-naive recipients who received an organ from seemingly "immune" donors (i.e., those with positive anti-HB surface, as well as core antibodies) has also been reported [152]. To prevent de novo hepatitis B in recipients of HBcAb + livers, most centers use antiviral prophylaxis [153].

Recurrent hepatitis by HBV usually occurs within the first 6 months of the transplantation, with the subsequent development of chronic active hepatitis within 9–12 months and of cirrhosis by 2–3 years after transplantation [150]. The strategy to prevent recurrent HBV infection after liver transplantation has evolved over the years. Monotherapy with hepatitis B immune globulin (HBIG) was used in earlier years. In one study [154], HBIG reduced HBV hepatitis recurrence, defined as reappearance of HBsAg after transplantation, from 76 to 19 %. Most centers administered HBIG after transplantation at 10,000 IU daily for 7 days starting immediately after transplantation, followed by reinfusion every 3–4 weeks for life to achieve and maintain serum levels of HBIG above 100 IU/L or above 500 IU/L to achieve more protective titer [88, 154, 155]. For HBV-viremic patients, the daily administration of the nucleoside analog lamivudine at a dose of 100 mg orally until the resolution of viremia was also used, as this drug has been shown to reduce the rate of HBV recurrence by its antiviral replication activity [88, 156]. Many centers used lamivudine before transplantation to control viremia, with the option of reintroducing the drug in those who develop breakthrough viremia despite HBIG after transplantation.

Long courses of lamivudine administration have been associated with emergence of lamivudine resistance. In one study, the rates of HBV DNA polymerase mutants were detected in 20 %, at a median of 26- and 38-month follow-up in pre- and posttransplant patients treated with lamivudine monotherapy [157]. Alternatively, lamivudine may be continued along with HBIG for those who were previously viremic, regardless of the result of surveillance HBV PCR after transplantation [88]. The combination of HBIG and lamivudine together allowed investigators to administer lower doses of HBIG and achieve lower rate of HBV recurrence at a reduced cost [158]. With the emergence of lamivudine resistance, alternative antiviral agents, such as adefovir and entecavir, have been used with or without HBIG administration [159, 160]. In recent years tenofovir and entecavir became the favorable antivirals because they are potent drugs with low rate of resistance [153, 161].

Despite the fact that these preventive strategies have been effective in reducing recurrence of HBV infection, occult infection of the graft can be demonstrated by molecular techniques in most HBV-related cirrhosis patients after liver transplantation; this suggests that long-term HBV prophylaxis is required in this population [162]. The type of prophylaxis depends on viral replication (i.e., detectable HBV DNA) before and at the transplant operation. Most investigators do not recommend antiviral suppression before transplantation when there is no evidence of viral replication, only when replication is documented [153, 161]. Lifelong combination of HBIG and antivirals is offered after transplantation in active viral replication. In the case of lack of HBV DNA, a short course of low-dose HBIG IM or IV with antivirals, followed by antiviral monotherapy, is recommended [153, 161].

14.5.7.2 Recurrent HCV

HCV recurrence is more problematic. As many as 90 % of liver transplant recipients who had HCV infection as the underlying cause of liver failure acquire detectable HCV RNA virus after transplantation. Seventy-five percent of these patients develop signs of liver damage, and 25 % go on to cirrhosis within 5 years [88, 163–165]. The risk factors for the early recurrence of HCV in allograft include the presence of HCV genotype 1B, steroid use, and treatment for acute rejection [165, 166]. Concurrent CMV infection may also increase the rate of recurrent HCV infection [166, 167]. Many studies show that combinations of interferon and ribavirin are effective for chronic HCV infection [168, 169]. A few investigators who treated patients awaiting liver transplantation with a combination of interferon and ribavirin demonstrated 30–50 % response at the end of treatment and sustained viral response around 20 %; this treatment was beneficial after transplantation in some of the patients who remained free of virus [170, 171].

The advent of direct antiviral agents in treatment of hepatitis C has changed the way hepatitis C liver transplant candidates and those after liver transplant is managed [172]. The availability of treatment protocols without interferon before and after transplantation made hepatitis C management more tolerable. According to the AASLD Practice Guidelines [173], treatment of HCV before liver transplantation leading to sustained virologic response (SVR) would usually prevent HCV recurrence after transplant. Treatment with sofosbuvir and ribavirin (up to 48 weeks), in patient awaiting transplant with MELD score up to 14 and Child-Pugh score up to 8, was well tolerated leading to SVR of 69 % after transplant [173]. Daily sofosbuvir and ribavirin in patients with compensated recurrent HCV after liver transplant lead to SVR of 70 % at 12 weeks [173]. According to the EASL preliminary guidelines [174], the following regimens (12–24 weeks) are recommended to liver transplant recipients with HCV recur-

rence: genotype 2, sofosbuvir and ribavirin; genotypes 1 and 3–6, sofosbuvir and daclatasvir ± ribavirin; and genotypes 1 and 4, sofosbuvir and simeprevir ± ribavirin [174]. With the emergence of all these new antiviral agents, it is expected to see significant change in the management of HCV in liver transplantation the next few years.

Currently, many centers employ the use of HCV-seropositive donors with favorable histology at liver biopsy for transplantation into recipients with HCV-related cirrhosis. Most cases demonstrate viral superinfection with nearly 50 % seroconverting to the donor genotype after liver transplant. In large series, these recipients of HCV-seropositive grafts have equivalent to slightly better outcomes than if they received grafts from naïve donor livers.

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15
Risks and Epidemiology of Infections After Intestinal Transplantation

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15.1 Historic Background

The field of intestinal transplantation has developed significantly since the first reports involving dogs in the late 1950s [1]. Although small series demonstrated some short-term success of intestinal transplantation in the 1960s [1, 2], long-term success was not reported until tacrolimus was introduced into clinical transplantation in 1990. Subsequently, dramatic and rapid progress in donor and recipient selection processes, evolution of surgical techniques, and enhancements in early perioperative care led to a significant improvement in early postoperative patient and graft survival in this challenging population. Key lessons were learned from the early failures in intestinal transplantation, as most graft loss was attributable to overwhelming rejection due to the inability to provide effective immunosuppression to this complex population in the face of such a lymphoid-rich, bacteria-containing graft [1]. Additional causes of early graft loss included ischemic injury of the allograft resulting in poor reperfusion and technical complications. Each of these early factors can be associated with infections occurring after transplant in association with severely damaged allografts rich in pathogens within the intestinal lumen [2]. Finally, morbidity and mortality were also encountered as a result of the mobilization and engraftment of donor-derived lymphocytes leading to graft-versus-host disease (GVHD) [3].

The introduction of tacrolimus immunosuppression in 1989 combined with the use of multidrug immunosuppressant protocols and donor and recipient anti-lymphocyte preconditioning markedly altered the results of intestinal transplantation. As a consequence, intestinal transplants are now performed worldwide with good overall results and long-term survival. Unfortunately, the high levels of long-term immunosuppression necessary to prevent and treat cellular and humoral rejection in ITx recipients have been an important contributor in the majority of deaths following intestinal transplant which have been directly related to infection and malignancy. Indeed, the future success of intestinal transplant relies on balancing the risks of immunologic complications associated with under-immunosuppression and the often fatal consequences of over-immunosuppression.

15.2 Patient Population and Risk Factors for Infection

Patients requiring intestinal transplantation have intestinal failure (IF) and require parenteral nutrition (PN) to maintain a normal state of fluid, electrolyte, and nutritional balance. IF is a result of either an anatomic loss of their intestine (e.g., congenital intestinal atresias or acquired disorders, such as volvulus, necrotizing enterocolitis, trauma, or vascular thrombosis) or poor function involving motility (e.g., intestinal pseudoobstruction, Hirschsprung disease), absorption (e.g., microvillus inclusion disease), encasing tumors (e.g., desmoids), or Crohn's disease. PN has changed the outcome for patients who have intestinal failure by effectively providing temporizing benefits parallel to those seen with hemodialysis in patients with kidney failure. However, long-term survivors of PN can experience morbidity associated with development of catheter sepsis, venous thrombosis-induced loss of vascular access, and PN-induced cholestatic liver disease. Liver disease and the secondarily dilated short gut seen with intestinal adaptation facilitate intestinal bacterial overgrowth, enteritis, bacterial translocation, the seeding of venous catheters, and line sepsis. Severe bloodstream infections can result in metastatic infectious foci, endocarditis, multisystem organ failure, and death. In addition, the infectious complications of IF may contribute to the development and progression of parenteral nutrition-dependent liver injury [4].

Life-threatening complications occur in as many as 20 % of patients on long-term PN. Without intestinal transplantation, patients with IF who develop complications of PN will die at a significant rate, with 1-year and 3-year survival rates of 84 % and 74 %, respectively [5, 6].

P. Ljungman et al. (eds.), *Transplant Infections*, DOI 10.1007/978-3-319-28797-3_15

Younger patients (<1 year old) have a higher risk of dying of infectious complications, possibly because they have an earlier onset of severe liver disease [4]. The most common infectious complication in patients with intestinal failure remains line sepsis, occurring with a frequency of 8.9 new catheter-related bloodstream infections (CRBSI) per 1000 catheter days [5]. Another single center study found an incidence of 3.49 septic episodes per child, with similar mortality risk at 2 years [7].

The "nonsterile" environment of the intestine defines the epidemiology of bacterial infections before and after transplantation. Many of these patients have a history of multiple catheter-related bloodstream infections and have been exposed to repetitive and/or long-term antibiotic therapy, which can facilitate bacterial overgrowth and translocation. The recurrent exposure to antimicrobial agents may lead to colonization and subsequent infection with antimicrobial-resistant bacteria and to the overgrowth of yeast and other fungi. Although prophylactic use of antimicrobial and ethanol lock therapy [8–10] or the use of antibiotic-coated catheters [11] may decrease the need for systemic antimicrobial therapy, catheter-associated bloodstream infections still represent a significant source of pre-transplant morbidity and mortality.

By definition, intestinal transplantation is conducted in a contaminated environment. The logistics of donor organ procurement does not allow for adequate bowel preparation prior to the procurement of the intestinal organs and although intestinal decontamination formulas are given to the deceased donor, effective mechanical cleansing is logistically impossible. Therefore, the succus entericus of the donor is transplanted with the intestinal allograft. Donor gastrointestinal flora in the lumen of the intestinal allograft may result in bacterial infections if significant preservation damage to the mucosa of the allograft intestine occurs due to ischemia–reperfusion injury. Subsequently, a similar breakdown of mucosal integrity may occur as a result of rejection which can also lead to bloodstream infections.

In addition to the usual sources of posttransplant sepsis (surgical site infections, abdominal abscesses, ventilator-associated pneumonias, etc.), many patients who have undergone a successful intestinal transplant continue to require central venous access for fluids, antibiotics, and antiviral therapy for a period of months after successful intestinal transplantation. While the central lines remain in place, the risk of catheter-related infections remains.

Pediatric intestinal transplant recipients present unique challenges. On average, the pediatric recipients are a young population, with a mean age of 7.2 +/− 6.5 years at the time of transplant [12] who may be "immunologically naive," thus having a higher risk of acquiring primary infections with *Cytomegalovirus* (CMV), Epstein–Barr virus (EBV), and other common community-acquired infections. Consequently, any requirement for higher baseline levels of immunosuppressive drugs to prevent rejection of the intestinal allograft predictably places them at an increased risk of developing opportunistic infections. This alone might explain the higher rates, prolonged disease states, higher morbidity, and increased mortality due to CMV and EBV infections that occur in these patients.

15.3 Anatomy, Pathology, and Pathogenesis of Infections

A number of different variations of intestinal transplantation can be carried out to ideally suit the anatomic and functional needs of the recipient; the etiology for intestinal failure dictates the type of intestinal allograft used. The need for associated stomach, pancreas, or colon in addition to the small bowel allograft is determined on the basis of the functional or vascular disease of those organs in the recipient. The diagnosis of end-stage liver disease as a consequence of TPN will determine the need for liver replacement. Consequently, intestinal transplantation procedures may provide the isolated intestine, the combined liver and intestine (with or without the pancreas), complete multivisceral intestinal allografts which include the entire gastrointestinal tract (i.e., stomach, duodenum, pancreas, and small bowel) along with the liver, and modified multivisceral grafts, which include the entire gastrointestinal tract (i.e., stomach, duodenum, pancreas, and small bowel) and exclude the liver [4]. Patient and graft survivals during the early posttransplant period vary according to the transplantation procedure. The best early survivals are associated with the isolated intestinal transplant, and the worst outcomes are observed in recipients of multivisceral allografts. The reason for this stems from the fact that recipients of isolated intestine allografts are relatively stable, when compared to patients with TPN-induced liver failure (portal hypertension, pancytopenia, coagulopathy) who will also require the larger composite grafts (liver/intestine or multivisceral grafts). The morbidity and mortality associated with outcomes after multivisceral transplantation stem from the difficulty of the resection portion of the operation and of the immediate posttransplant management. The risk of infection inherently determines short-term and long-term survivability.

Damage to the protective barrier of the intestinal mucosa can occur early from ischemia and reperfusion damage and later from rejection or GVHD of the intestinal allograft. Factors that determine risk for ischemia and reperfusion of the intestinal allograft are similar to those seen with other solid organs; they focus on a history of cardiac arrest and on the subsequent hemodynamic instability of the cadaveric brain-dead donor.

Prolonged episodes of cardiac arrest and hypotension and the need for multiple vasopressor drug therapy to maintain the blood pressure in the donor may herald the inevitable ischemia of the intestinal allograft. Such episodes of ischemia may be reflected in donor liver functions (elevated transaminase and bilirubin levels).

After the implantation of the intestinal allograft, ischemia/reperfusion syndromes may develop and be manifest by hemodynamic instability, fibrinolysis, and bleeding. Serial biopsies of intestinal allografts at the time of surgery and in the postoperative period reveal the severity of damage and the potential for recovery. In this setting, bacteria within the succus entericus brought with the intestinal allograft can traverse the intestinal epithelium and enter the splanchnic venous system. With isolated intestinal transplantation, the consequences of such an efflux of bacteria and endotoxin will depend on how the graft is drained. If drained into the recipient's portal or superior mesenteric vein, transient elevations in liver function tests may occur. If it is drained into the systemic circulation via the recipient's inferior vena cava, more significant manifestations of bacteremia may be seen, including adult respiratory distress syndrome (ARDS). In addition to the potential deleterious effects of luminal bacteria, work is being done to determine the relationship between the intestinal allograft microbiome, pathologic bacterial overgrowth and intestinal rejection [13, 14].

As the mucosal barrier is broken with immunologic injury, clinical sepsis can also accompany severe rejection in which breakdown of the allograft mucosal barrier occurs due to damage from native immunocytes of the recipient. More commonly, rejection episodes tend to be mild to moderate, without such infectious consequences; however, acute rejection should be considered as a potential etiology of any bacteremia with enteric organisms. The overall incidence of rejection of the intestinal allograft has been high (greater than 85%), with the average patient experiencing between one and five episodes of rejection per graft [6, 12, 15]; more recent progress in immunosuppression management has introduced the ability to minimize immunosuppressive load, thereby resulting in less rejection and consequently less infection. This high frequency of rejection of varying severities likely accounts for the high frequency of bloodstream infections seen in intestinal transplant recipients. The clinical signs of rejection include abdominal pain, distention, diarrhea, nausea, vomiting, and fever; however, these can also suggest the presence of a concomitant bacterial or fungal infection. The diagnosis of rejection is based on the findings from endoscopic biopsies of the intestinal allograft mucosa. Such confirmation is critical, particularly in long-term patients who present with minimal symptoms, and fever as opportunistic infections, such as CMV and EBV, can produce similar symptoms. In addition, episodes of enteritis secondary to infections with EBV and CMV may also be accompanied by damage to the mucosal barrier and associated bloodstream infections with bacteria and fungi.

Although rare, technical complications associated with intestinal allograft implantation complicate 7.6% of cases [16]; these include intestinal anastomotic leaks resulting in infectious peritonitis, vascular complications such as arterial thrombosis with consequent graft ischemia, and intestinal volvulus. These technical issues are certainly associated with severe septic and infectious complications and prompt surgical correction is required for survival.

15.4 The Intestinal Transplant Timetable

The unique timetable for infectious complications after intestinal transplantation is noted in Table 15-1. Bloodstream infections consequent to surgery-related (i.e., donor and implantation operation) and catheter-related infections continue to occur for at least the first month after transplantation. Because the mucosal barrier of the intestinal allograft is continuously exposed to the external environment, any breaks in the mucosal surface may result in transient bacteremia. Furthermore, indwelling intravenous catheters may be required for up to a year or longer after transplantation, placing these recipients at a higher risk of bacteremia. The high risk of CMV and EBV diseases carries a proportionately higher morbidity and mortality as well. The baseline immunosuppression levels—higher than those for other solid organs—that are required for a long term to maintain the intestinal allograft place these patients at a higher risk for symptomatic disease from these pathogens. Children may also be serologically naive for CMV and EBV, thus increasing the already high risk for disease. The chronic, higher level of immunosuppression required by these patients also raises the risk for severe infections from community-acquired pathogens (e.g., influenza, respiratory syncytial virus [RSV], adenovirus, streptococcal pneumonia, and *Pneumocystis jiroveci* pneumonia). However, despite high baseline immunosuppression, most intestinal transplant recipients who develop these infections tolerate them quite well because of their improved overall health status.

Patient and graft survival after intestinal transplantation is inherently related to the risk of rejection, the need for antirejection therapy, the high levels of baseline immunosuppression, and the risk of posttransplantation infections. In addition, the relationship between patient and graft survival is inherently dependent on the reversibility of the

Table 15-1. Timetable for infections after intestinal transplantation

Early (0–90 days after transplant)	Late (3–6 months after transplant)
Surgical site—usually intra-abdominal	Catheter-associated bacteremia
Catheter-associated bacteremia	Rejection-associated bacteremia
Pneumonia	EBV/PTLD
Rejection-associated bacteremia	Opportunistic infections
Donor derived infections	Candidemia
Early viral infections (adenovirus, influenza)	Pneumocystis jiroveci
CMV	Herpes simplex
	Varicella zoster

Abbreviations: *CMV Cytomegalovirus*, *EBV* Epstein–Barr virus, *PTLD* post-transplant lymphoproliferative disease.

type of transplant performed. For example, if a patient who has undergone an isolated intestinal transplant develops a life-threatening complication of the intestine or immunosuppression (e.g., uncontrollable rejection or posttransplant lymphoproliferative disorder [PTLD]), the intestinal allograft can be safely removed and the immunosuppression discontinued. Unfortunately, patients who have undergone combined liver and intestinal transplants have a much poorer outcome after the removal of the intestine and discontinuation of immunosuppression with the rapid development of allograft liver disease and death. Until recently, this was responsible for a progressively declining survival curve up until 3–4 years, at which time it plateaus (Figure 15-1). This curve contrasts with that of recipients of liver or kidney allografts in whom, after the first posttransplantation year, the baseline levels of immunosuppression are lowered, with a declining risk of rejection or infectious complications in the later posttransplant years. This resulted in a higher rate of survival at all posttransplant times.

Furthermore, the intestine is a large lymphoid-rich organ, the immunologic role of which cannot be underestimated.

15.5 Early Infections After Intestinal Transplantation (<90 days)

Although clinically useful to diagnose and empirically treat suspected infections after transplant, the timeline for infectious complications is not absolute. In addition, certain pathogens can be either donor derived or present in the recipient at the time of transplant, making the pre-transplant

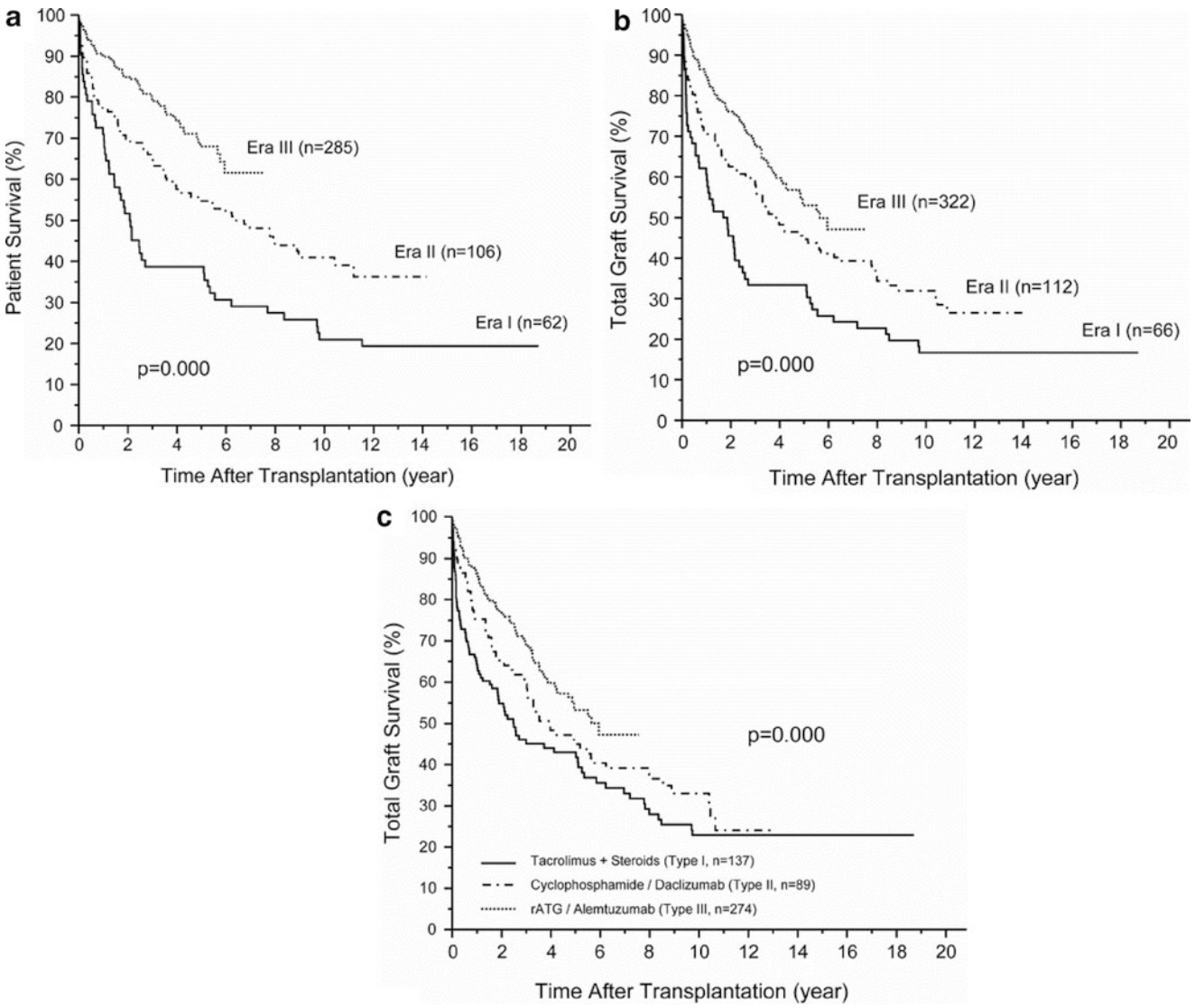

FIGURE 15-1. Graft and patient survival based on the era of immunosuppression at the time of transplant. From Abu-Elmagd KM, Costa G, Bond GJ, et al. Five hundred intestinal and multivisceral transplantations at a single center: Major advances with new challenges. Ann Sug 2009; 250: 567–81.

screening of both the donor and recipient imperative [17]. Even minor and common viral infections in the pre-transplant immunocompetent host can blossom into severe and life-threatening infections after preconditioning of the recipient. In addition, infectious complications in the early period lead to significant increases in the length of stay [18]. Likewise, unsuspected infections in the donor can rapidly manifest in the immunosuppressed recipient. Thus, although these timelines are useful for discussion, it should be kept in mind that common entities causing perioperative infections can also be acquired late after transplant.

15.5.1 Bacteremia and Fungemia

Based on the aforementioned clinical characteristics of intestinal transplantation, it is not surprising that the rates of bacterial and fungal infections observed after intestinal transplantation are higher than those reported for patients undergoing other types of solid organ transplantations, such as those for the liver, heart, or kidney.

Bacterial infections account for close to 60 % of all infections after intestinal transplantation [19]. Bacteremia has been shown to be the most common type of infection identified in children undergoing intestinal transplantation, with 69 % of children developing a bacteremia in the first postoperative year [20] and at a rate of approximately 1.4–1.7 episodes per patient per year. Similar infectious morbidity and frequency have been observed in adult recipients, with a rate of 1.55 episodes per patient after intestinal transplantation [17, 21]. The incidence of bacteremia after intestinal transplant does decrease over the course of the first year, declining from ~80 % in the first 8 weeks to roughly 3 % per month after the first 6 months [22–24].

The overall incidence of bacteremia after intestinal transplantation can be higher than 60 %; enteric organisms, including *Enterobacter*, *Klebsiella*, and *Pseudomonas* species are the most frequent organisms recovered [25]. These gram-negative organisms reveal a trend toward polymicrobial infection with multiple-antibiotic-resistant organisms, but other combinations of isolated gram-negative Enterobacteriaceae and coagulase-negative staphylococci also occur. While disruptions of the mucosal barrier of the allograft are frequently present, an obvious source of bacteremia is often not identifiable. This may be because the majority of the surface area of the intestinal allograft is not visible, as endoscopy reveals only the most distal or proximal portions of the intestine.

Although abnormal allograft histology does not have to be accompanied by bacteremia, in the presence of significant gastrointestinal pathology, enteric pathogens historically have been recovered in more than 50 % of cases [25]. Consequently, correlations between bacteria identified in the stool and those identified in the blood are high. The authors believe that bacterial overgrowth, which is defined as a bacterial count of more than 10^9 colony-forming units (CFUs) per milliliter of stool, may be a determining factor in the development of translocation in the setting of intestinal mucosal damage.

This association of enteric bacteremia (and CLABSI) and loss of mucosal function leads to the clinical caveat that if enteropathogens are identified in blood culture specimens, consideration should be given to performance of an endoscopy to identify the presence of underlying pathologies, such as rejection or enteritis (i.e., CMV or EBV). The treatment for suspected bacteremia should take into account the antimicrobial susceptibility patterns of bacterial pathogens associated with prior episodes of infection both before and after transplantation. Changes to the immunosuppressive drug strategy inherently parallel the treatment of any infection in patients after ITx. If the cause of mucosal breakdown and translocation is viral enteritis (CMV, EBV, adenovirus), judicious reduction of the immunosuppression is warranted. Paradoxically, in the face of allograft rejection, higher levels of immunosuppressive medication and antirejection therapy will be required.

Finally, it is worth noting that fungemia is also seen at higher frequencies in ITx recipients compared with recipients of other types of solid organ transplantation, occurring in 23–59 % of intestinal transplant recipients [26–28]. The majority of those infections were found to be due to *Candida* spp., accounting for 80–100 % of infections, and early candida infections are often associated with intra-abdominal abscess or intestinal leak [26]. Although *C. albicans* remains the dominant species isolated (37–46 %) after intestinal transplant, *C. glabrata* and *C. parapsilosis* are also common, representing 25 % and 13 % of isolates, respectively. There is some debate as to whether candidal infections have a negative impact on survival after intestinal transplant, with one study showing a significant decrease in survival with only *C. glabrata* species [27]. Antibiotic therapy and preoperative PN use are significantly associated with candidal infections in pediatric intestinal recipients.

In addition to bacteremia, significant morbidity is associated with respiratory tract infections in the early postoperative period, accounting for roughly 15 % of bacterial infections in this population. Health-care-associated respiratory infections account for the majority of these infections, with a similar microbiology to other hospitalized populations [19, 22].

15.5.2 *Cytomegalovirus* Infection

The success of clinical intestinal transplantation after 1990 under immunosuppression with tacrolimus was accompanied by an unexpectedly high prevalence and marked severity of CMV disease. These higher rates and levels of disease severity were attributed to the higher burden of immunosuppression required to prevent rejection. The overall incidence

of CMV infection at the authors' center and throughout the International Intestinal Transplant Registry has been reported to be as high as 20 % [29]. Fortunately, the incidence of CMV disease has decreased as experience with viral monitoring, immunosuppressive strategies, and preemptive treatment therapies has evolved. Our most recent series of intestinal transplants was found to have a 7 % incidence of CMV disease, compared to a 35 % incidence in patients transplanted using different immunosuppressive and preventative strategies [16].

Analysis of this historical high rate of CMV disease (38 %) in adult recipients after intestinal transplantation at a single center stratified the rates of CMV disease by the serologic status of CMV in the donor and recipient (D/R) as follows: D−/R−, 0 %; D−/R+, 50 %; D+/R+, 50 %; and D+/R−, 75 % [30]. Similar rates of CMV disease have been seen in these groups in more recent studies, and in general, patients can be stratified into high-risk (D+/R−), low-risk (D−/R−), and intermediate-risk groups, based on serologic data. These groups can then be used to develop strategies for CMV disease prevention.

In a recent international survey of intestinal transplant centers, 40 % of programs used universal ganciclovir or valganciclovir prophylactic therapy for prevention of CMV disease in high-risk (D+/R−) populations. In addition, 35 % of programs administered high-titer CMV-immunoglobulin (CMV-IVIG) prophylaxis to the high-risk group. Recipients who were CMV positive at the time of transplant were more frequently treated with frequent monitoring for viremia and preemptive therapy; however, 12 % of centers administered prophylactic antiviral therapy and 20 % of centers still utilized CMV-IVIG as part of the prophylaxis for CMV. Despite these strategies, 20.3 % of patients still developed CMV infection [31].

CMV has a propensity to involve the gastrointestinal tract, particularly the intestinal allograft. Indeed, CMV enteritis accounted for more than 80 % of all episodes of illness. Risk factors, such as rejection and the need for steroids or OKT3, associated with the development and/or recurrence of CMV disease in this patient population were similar to those seen with other solid organ transplants, but they had a higher impact [32]. Interestingly, rejection was not found to be a risk for CMV disease in a recent study [33] and invasive CMV enteritis has been seen in patients without associated CMV viremia [34].

Although CMV disease historically occurred at a higher frequency, it remained a treatable disease, with a similar 1-year survival rate among patients with or without a history of CMV. Because ganciclovir prophylaxis had been ineffective in preventing the disease in children and adults in the 1990s, several centers would avoid CMV-seropositive donor organs in CMV-negative candidates awaiting isolated intestinal transplantation. This policy did not generally apply to patients waiting for combined liver and intestine transplants; due to the excessive mortality risk, end-stage liver disease places these patients at a higher risk of dying while waiting for organ allografts. However, the availability and effectiveness of prophylactic strategies, as well as changing patterns of CMV disease potentially associated with the use of induction immunosuppressive therapies, may affect the prevalence and outcome of CMV disease. Indeed, most recent studies have confirmed almost nonexistent mortality from CMV disease, even in high-risk patient populations [16, 33].

The experience with CMV disease in the pediatric population had been similar, although less morbidity and minimal mortality were seen [35]. The intestinal allograft was infected with CMV in more than 90 % of patients experiencing CMV disease. In addition, the native intestinal tract was infected in 20 % of CMV-affected children. Recurrent and persistent CMV disease was common. As in adults, the D+/R− group of recipients had the highest frequency and morbidity. However, rates of CMV disease have declined with aggressive use of CMV-preventive strategies combined with modifications in immunosuppressive strategies. Experience from our center in pediatric ITx recipients receiving induction therapy with thymoglobulin identified that only 1 of 36 children developed CMV disease [12].

Ganciclovir remains the primary agent used to treat CMV disease, though there are several centers that utilize oral valganciclovir for both prevention and treatment of CMV [31]. Although this remains an attractive strategy, it should be done with some degree of caution and close monitoring, as there has been a reported increase in CMV tissue invasive disease in patients receiving valganciclovir, compared to intravenous ganciclovir [36]. The disadvantage of prolonged treatment with antiviral agents remains the significant adverse effects seen with these agents, primarily marrow suppression (anemia, leukopenia, and thrombocytopenia) and nephrotoxicity (survey paper). In addition, controversy exists regarding the proper dose of oral valganciclovir in the pediatric population [37, 38]. Strategies for the treatment of CMV infections also need to prevent recurrent infection, which can occur in up to 85 % of cases. This risk may be minimized by utilizing prolonged prophylaxis after treatment of the primary infection with either ganciclovir or oral valganciclovir.

The management of CMV disease with intravenous ganciclovir, alone or in combination with CMV-specific hyperimmune globulin, has resulted in clinical cures in 90 % of children with CMV disease with no alteration in long-term patient or graft survival [35]. The addition of CMV-IVIG for the treatment of CMV is generally recommended, although little data exists to support its use. Despite a lack of strong evidence to support its use, immunoglobulin administration remains a standard therapy in several treatment protocols for prevention and treatment of CMV disease in intestinal transplantation [31, 39].

Isolated cases of disseminated CMV in intestinal recipients have been reported that correlate with clinical manifestations suggestive of ganciclovir resistance; these were

accompanied by progressive rises in CMV antigenemia despite an appropriate regimen of ganciclovir and CMV-IVIG. Foscarnet provided eventual resolution in these cases [35]. Ganciclovir-resistant CMV infection occurs in up to 15 % of patients after lung transplant and has been found to be due to mutations in the CMV UL 97 or UL54 genes, which respectively encode the viral DNA phosphotransferase and the CMV DNA polymerase. Invasive infections with ganciclovir-resistant CMV are associated with 25 % mortality despite treatment with IVIG and foscarnet and a 78 % rate of foscarnet-induced toxicity [40]. Case reports using the prodrug leflunomide suggest its inclusion as a potential tertiary agent for treatment of resistant CMV infection [41]. In addition to drug therapy, studies in the HSCT literature are emerging that successfully utilize adaptive immunotherapy to treat CMV disease resistant to multidrug therapy [41]

In summary, CMV has been a frequent and important cause of morbidity after intestinal transplantation. CMV has a unique propensity to involve the intestinal allograft and the native gastrointestinal tract, which makes surveillance practical. At present, the use of long-term prophylactic therapy (3-month to 6-month courses) with ganciclovir (either IV or oral valganciclovir) in combination with CMV-specific hyperimmune globulin should be considered for CMV +/– patients [42]. The use of "hybrid" strategies using shorter courses of chemoprophylaxis followed by serial measurements of the CMV viral load in CMV-seropositive recipients may be effective but data are needed before this strategy can be widely recommended [42]. Prompt diagnosis and treatment of CMV viremia decreases the rate of invasive disease, though little data exists to determine the proper duration of treatment to avoid recurrent disease. Ganciclovir-resistant CMV disease exists and can be associated with significant morbidity and mortality in the lung transplant population. The emergence of immunotherapy as a potential modality for treatment of resistant disease may improve the results in these patients.

15.5.3 Adenovirus

Adenovirus is a ubiquitous DNA virus that can be either transmitted from the donor or can be acquired from the community. Like EBV, adenovirus can remain latent in the lymphoid tissue, allowing for its transmission and also accounting for the possibility of reactivation after transplant or during periods of augmented immunosuppression. Although the intestinal allograft is the most common site of infection, adenovirus can disseminate and can also infect a large variety of organs, including the brain, liver, lungs, and pancreas. The intestine was infected in 83 % of cases in a recent study [43]. Adenoviral infection is most common in younger recipients and is generally thought of as an "early" pathogen, with almost 70 % of cases occurring in the first 6 months after transplant. Overall, adenovirus infection has been observed frequently in pediatric recipients of intestinal transplantation with rates of adenovirus infection in this population ranging from 20.8 to 100 % [17, 21].

Adenoviral enteritis generally presents as an osmotic diarrhea, often without associated fever or systemic illness. The gross morphology of infection is one of hyperemic mucosa and ulceration, often difficult to distinguish from cellular rejection [44]. Histologically, there is severe villous injury and characteristic cytopathic adenoviral inclusions in the villi. Crypt apoptosis can also be seen in cases of severe adenoviral enteritis, potentially confusing the underlying diagnosis difficult [45]. Immunohistochemistry allows for better delineation of the inclusions in these cases and serum and tissue DNA polymerase chain reaction can also be used, if needed to confirm the diagnosis [44]. Adenovirus can also present as an invasive disease [46]. Risk factors for invasive disease include failure to clear virus, isolating virus from more than one site, and intensified immunosuppression [21].

It is very difficult to presumptively diagnose infection due to adenovirus in ITx recipients, as fever, hepatitis, and pneumonia may be caused by a variety of other pathogens. In addition, high stool output after IT is nonspecific and can also occur with rejection. The presence of high-grade fevers and symptoms suggestive of adenovirus infection should prompt serial cultures for viruses (including adenovirus) or PCR of the blood and evaluation of graft biopsies. Unexplained hepatitis should warrant consideration of a liver biopsy. Similarly, an increase in stool output, with or without fever, should prompt endoscopic evaluation of the intestinal allograft. Histologic examination for the presence of adenoviral inclusions as well as the use of immunohistochemical stains of biopsy specimens from either site should be undertaken to help confirm this diagnosis.

Unfortunately, there is no definitive treatment for adenoviral infection at this time [47]. The treatment of adenoviral infections is thoroughly discussed elsewhere (Chap. 34). The most important component of therapy is supportive care along with a judicious and carefully monitored reduction in immunosuppression. The role of antiviral agents is unproven, although there is increasing experience and enthusiasm for the use of cidofovir [48, 49].

15.6 Late Infections After Intestinal Transplantation (>90 days)

15.6.1 Epstein–Barr Virus-Induced Infection and Posttransplant Lymphoproliferative Disease

EBV is a gamma herpesvirus that infects the B cell population. EBV has a 95 % seroprevalence in the adult population worldwide, with roughly 50 % of the population in developed countries being exposed by the age of five. EBV is

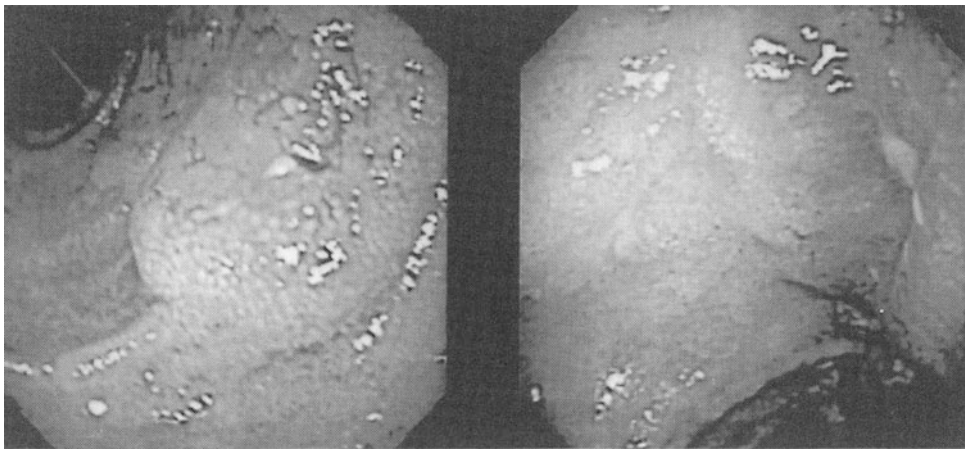

FIGURE 15-2. Endoscopic image of posttransplant lymphoproliferative disease lesion in an intestinal allograft.

directly responsible for the development of the clinical syndrome of infectious mononucleosis in immunocompetent people and also leads to EBV-driven diseases in organ transplant recipients and other immunosuppressed individuals [50]. The early experience in intestinal transplant under either cyclosporine- or tacrolimus-based immunosuppression identified EBV disease and PTLD as a major cause of morbidity and mortality. The ability to directly monitor for EBV viral replication with PCR allowed us to better define the spectrum of EBV infection and disease in recipients of intestinal transplantation and devise methods to avoid the development of lymphoma in these patients. EBV infection is often discovered as asymptomatic viremia. If unrecognized and left untreated, EBV viremia can progress in immunosuppressed hosts to invasive EBV disease and can then potentially further progress to polyclonal and potentially monoclonal PTLD including lymphoma. EBV infection can also present clinically as a febrile syndrome, nonspecific lymphadenopathy mimicking mononucleosis.

In the early era of intestinal transplant, OKT3-based immunosuppression and the lack of EBV-monitoring protocols lead to the development of PTLD in 25 % of recipients [51]. With the development of preemptive therapy protocols and refined immunosuppressive protocols, the PTLD incidence and mortality were significantly reduced to 7 % [51].

Similar to CMV, the most common location for invasive EBV disease is the allograft itself, with the intestine being involved in 71 % of the recipients. Historically, the diagnosis of EBV infections and of PTLD is typically in the first year after transplant, with the average time from transplant to the diagnosis of PTLD in pediatric ITx recipients being 9 months [52, 53]. However, later cases do occur and PTLD is diagnosed after the first year in roughly 30 % of cases [52, 53]. Prior to monitoring protocols, EBV disease was frequently found with endoscopy or a computed tomography after nonspecific abdominal findings or peripheral lymphadenopathy in an ill patient [12]. The pattern of EBV disease in intestinal

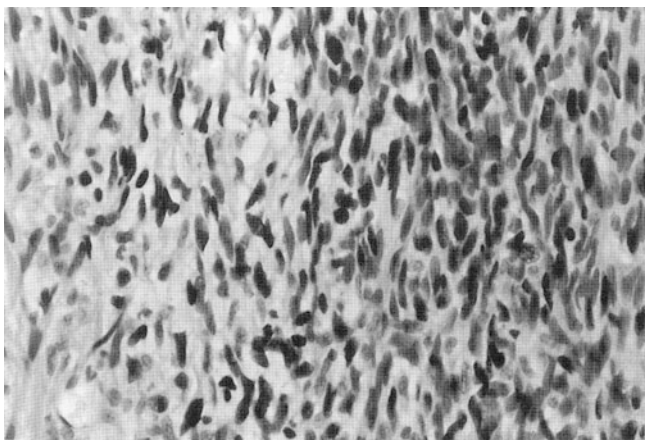

FIGURE 15-3. Posttransplantation spindle cell lesion (PTSD).

transplant recipients is similar to patterns historically seen with other solid organs, in which a nonspecific viral syndrome may eventually progress to PTLD. It can present clinically as a febrile syndrome, nonspecific lymphadenopathy mimicking mononucleosis, PTLD, or lymphoma. Similar to CMV, the most frequent site of involvement is the intestinal allograft; however, the native gastrointestinal tract is also frequently involved. The classic presentation is lymphadenopathy (although this may be limited to intra-abdominal location), blood in the stools, and an endoscopic finding of nodular ulcerated tumors in the intestinal allograft or the native gastrointestinal tract (Figure 15-2).

The histology of EBV disease is also similar to that described in other solid organ transplant recipients, with both polymorphic and monomorphic disease and variations in clonality being seen. Of interest, however, is the fact that the authors have cared for several patients who developed EBV-associated spindle cell tumors (Figure 15-3). These children had previously experienced an episode of EBV-associated PTLD. The EBV-serologic status prior to transplantation is unique to this population because, in con-

trast to other organs where EBV seronegativity is a major risk for EBV disease and PTLD, PTLD has been observed in seropositive patients as frequently as in seronegative patients (20.7 % vs. 19.4 %) [53].

The high frequency of, and the mortality associated with, EBV disease prompted the development of a prevention and preemptive treatment strategy for EBV infection in intestinal transplant recipients and has been a useful adjunct in the management of patients with established disease as well. In 1994, the authors began monitoring the EBV viral load in the peripheral blood using a quantitative competitive polymerase chain reaction (QC/PCR) assay. This monitoring was coupled with a preemptive therapy (PT) strategy in an effort to diagnose early EBV infection rather than after established disease or PTLD is present. This strategy included the use of ganciclovir and intravenous immune globulin and the judicious weaning of immunosuppression. The management of EBV disease and PTLD in intestinal transplant recipients is more complex than that with liver transplant recipients, because significant decreases or withdrawal of immunosuppression, which are the mainstay for management in other solid organ recipients, can result in severe breakthrough intestinal allograft rejection. This strategy is even more challenging as ITx recipients can present with evidence of concomitant EBV disease and rejection. Thus, the morbidity and mortality in the setting of PTLD can stem from the lymphoproliferative process, concomitant infections with CMV and bacterial pathogens, and/or severe intestinal allograft rejection [52, 53].

Patients with active EBV disease typically have an elevated EBV viral load in the peripheral blood; however, elevated loads may be present in otherwise asymptomatic individuals. These individuals are amenable to PT with intravenous ganciclovir (10 mg/kg/day), CMV IVIG (300 mg/kg every 2 weeks), and possible reduction in immunosuppression. At our institution, this therapy is instituted for elevated and/or rapidly rising viral loads. The exact level of EBV load to initiate PT will vary according to which assay is used to measure the load. In addition, we have typically initiated PT at very low loads for EBV-seronegative recipients experiencing primary infection while we have used cutoffs close to levels where EBV disease and PTLD are observed for those who are EBV seropositive prior to transplant. Although the effectiveness of each of the individual components of this strategy remain unproven, the combined use of this approach along with evolutions in immunosuppression algorithms for these patients has resulted in decreases in the incidence, morbidity, and mortality attributable to EBV disease and PTLD in this population [53].

Interestingly, this protocol also led to the recognition of a subgroup of children (roughly 20 % of children) who fail to resolve their EBV viremia. This results in the development of a high-load carrier state, in which patients have a significant EBV viremia (>16,000 EBV copies/mL whole blood at our institution) on >50 % of samples for more than 6 months, following either asymptomatic viremia or resolution of invasive EBV disease/PTLD. Management of these patients is challenging as 37 % will develop EBV disease with PTLD being diagnosed in 11 %, despite appropriate preemptive treatment and reduction in immunosuppression [54, 55].

In addition to judicious reduction in immunosuppression and institution of antiviral therapies, one must carefully monitor patients with EBV viremia for the development of invasive disease and PTLD. Because the lesions most frequently are found in the intestine, any patient with symptoms should have a lower and/or proximal endoscopy, followed by CT of the neck, chest, abdomen, and pelvis. Evidence supports a potential role for PET scans for the diagnosis and follow-up of PTLD in selected patients, though specific guidance on its use is not available [56].

Biopsies of suspicious lesions will provide histologic confirmation of the diagnosis of PTLD; however, the use of immunohistochemical stains for the presence of EBV (Epstein–Barr early RNA stain [EBER stain]) is a helpful adjunct for distinguishing EBV-infected cells from nonspecific lymphocytic infiltrates. Such EBV infiltrates may also produce apoptosis in the intestinal crypt epithelium, thus mimicking rejection. In addition, biopsies of lesions should be evaluated for the expression of the cell marker CD20 that predicts the response to potential treatment options.

The historic management of EBV disease and PTLD has centered on significant reductions in, or withdrawal of, immunosuppressive medications, thus allowing the immune system to develop a cytotoxic T-cell lymphocyte response to control the infectious process. With intestinal transplant recipients, such manipulations of immunosuppressive drugs have resulted in high rejection rates, complicating the management of EBV disease and PTLD. More recent immunosuppressive management strategies using antilymphocyte globulin induction and tacrolimus at low levels (10 ng/mL) and no steroids have allowed for significant improvement in the incidence and severity of rejection and also the ability to minimize immunosuppression in a similar fashion as with other organs. This has resulted in improved survival and also in a significantly lower rate of CMV and EBV/PTLD [12] (Figure 15-4). The majority of patients will demonstrate some evidence of a clinical response within 2–4 weeks after modulation of medications. The impact of EBV viral load monitoring on the incidence and outcome of EBV disease and PTLD cannot be overemphasized. Rates of EBV disease and PTLD using this approach have dropped dramatically when compared to a cohort of children undergoing intestinal transplantation at the authors' institution prior to the availability of this approach [54]. Therefore, early diagnosis does affect the outcome of EBV disease, and judicious reductions of immunosuppression in this setting allow resolution with a lower incidence or absence of rejection of the intestinal allograft.

The mortality of this disease during the authors' initial experience was pervasive (50 %), principally due to the asso-

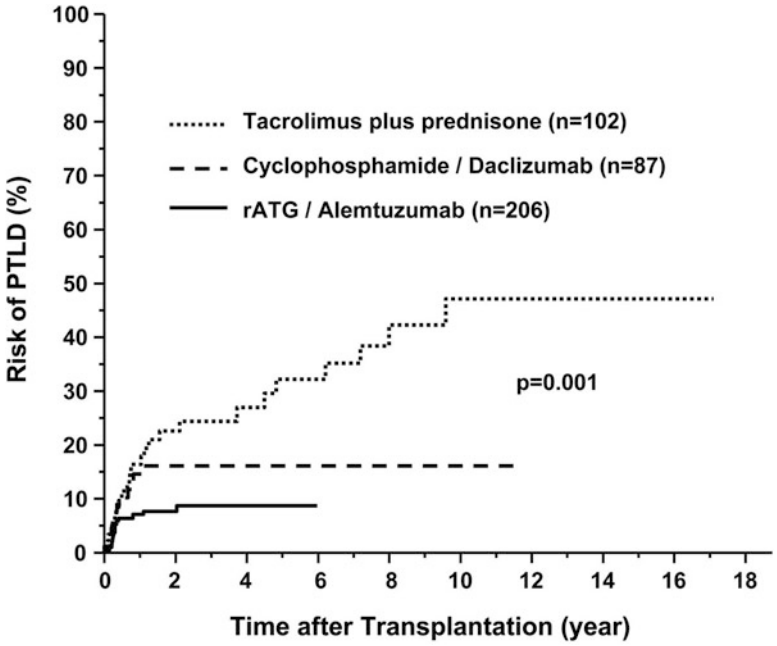

FIGURE 15-4. Cumulative risk of PTLD in three eras of immunosuppression. Note the striking differences in PTLD rates in patients receiving rATG preconditioning (*solid line*). From Abu-Elmagd K, Mazariegos G, Costa G, Soltys K, et al. Lymphoproliferative disorders and de novo malignancies in intestinal and multivisceral recipients: Improved outcomes with new outlooks. Transplantation 2009; 88:926–34.

ciated rejection during the treatment for PTLD. During this early experience, the results of treatment of lymphoproliferative disorder were further limited by the fact that 30 % of the surviving patients experienced chronic and/or recurrent episodes of EBV disease after successful therapy [52]. This contrasts sharply to the recurrence rate of 5–10 % seen with other solid organ transplants. This likely results from the ongoing need of the intestinal transplant recipient to continue with high baseline levels of immunosuppressive therapy, which limits the body's ability to generate a cytotoxic T-cell lymphocyte response against EBV. Of note, evolutions in the management of ITx recipients, including evolving immunosuppression strategies and the use of EBV load monitoring, have resulted in a marked improvement in the outcome of PTLD [51]. Unfortunately, this will not be adequate treatment in some PTLD lesions that are no longer under control of EBV and behave more like true malignancies [50]. The authors recommend a trial of immunosuppression reduction unless there is concurrent rejection or histologic evidence of true malignancy. In addition to IS reduction, antiviral and immunomodulating medications can be used as potential therapy, with failure leading to the use of cytotoxic chemotherapy.

Newer therapies for the treatment of EBV disease and PTLD are also showing promise [57, 58]. Rituximab is a chimeric humanized antibody against the B-cell antigen CD20. Studies have demonstrated variable success in using rituximab in treatment of PTLD that has not responded to IS reduction. Studies in children have shown good short-term response rates of 80 %, though there is a significant recurrence rate of 25 % [58]. Rituximab has become a key component of treatment of CD20-positive PTLD in some intestinal recipients, especially those with concurrent rejection or those not responding to the combination of immunosuppression reduction and antiviral/immunoglobulin therapy. Vigilant posttreatment follow-up is required as recurrence is possible and occurred in roughly 20 % of responders in one large French study, at a median of 7 months after treatment. In addition, a significant percentage of patients will develop hypogammaglobulinemia and should be appropriately treated [58]. Additionally, encouraging results have been obtained using a combination of low-dose chemotherapy and rituximab in the treatment of PTLD in setting of solid organ transplant. Studies have shown 2-year relapse-free survival rates of 70 % [59]. While the use of EBV-specific cytotoxic T-cells (CTL) to treat EBV-related PTLD (which has shown to be effective in bone marrow recipients) is of interest, experience to date has not been able to demonstrate definitive success in SOT recipients. As an alternative approach, Haque and colleagues have developed a bank of 100 EBV-specific CTLs, using healthy blood donors in the UK. Using this bank, the authors used HLA "best fit" CTLs in a phase II multicenter trial to treat PTLD that was refractory to other methods with 52 % overall response rate. Of those patients that had a response, an impressive 82 % were complete [60] with documented long-term survival in responders [61]. Finally, transplant physicians should also recognize the importance of allograft enterectomy to allow for complete withdrawal of immunosuppression in cases where such an approach can be taken (isolated intestinal transplant), especially for those not responding to first- or second-line therapies.

15.6.2 Respiratory Illnesses

Respiratory viruses remain a major source of morbidity and mortality in immunocompromised organ transplant recipients (Table 15-1). The majority of lower respiratory tract viral infections (LRTRVI) are caused by RSV, influenza, parainfluenza, adenovirus, and rhinovirus. Fortunately, advances in qualitative and quantitative RT-PCR technology have allowed for the rapid and reliable detection of specific LRTRVI pathogens with 95 % overall sensitivity and specificity [62].

In a recent retrospective review of 25 children who had undergone intestinal and/or liver transplant, the Miami group reported a high rate of rhinovirus as an etiology of LRTRVI. Although the overall mortality associated with LRTRVI was from 13 %, no children with rhinovirus succumbed to the disease. Interestingly, RSV infection was associated with a 40 % mortality, and was the direct cause of death, despite reduction in immunosuppression and administration of palivizumab [63]. Due to the potential morbidity and mortality associated with RSV LRTRVI [64], some experts recommend immunoprophylaxis with an RSV-specific monoclonal antibody (palivizumab) for children less than 1 year of age who are transplanted or are receiving antilymphocyte treatment during RSV season. Treatment of RSV is limited, though careful reduction in baseline immunosuppression should be considered if clinically warranted. Aerosolized ribavirin is currently approved for the treatment of severe RSV LRTRVI and is generally used in combination with palivizumab, though data supporting this are limited. Intravenous ribavirin has also been used for treatment; the benefit of this agent must be weighed against the potential for the development of complication, specifically hemolytic anemia [65]. An in-depth discussion of other respiratory viruses (influenza, parainfluenza, and coronaviruses), their treatment, and prevention with vaccination strategies before and after transplantation can be found elsewhere (Chaps. 30 and 32). However the authors would stress the importance of *avoidance* of infection with well-thought-out policies regarding timing of vaccination and/or immunoprophylaxis in this challenging population.

Pneumocystis jiroveci also represents an important pathogen to be considered in intestinal transplant recipients. Based on older studies done prior to the routine use of prophylaxis, up to 15 % of organ transplant recipients experienced an infection with *Pneumocystis*. The clinical course of *Pneumocystis jiroveci* pneumonia is generally one of dyspnea and hypoxia out of proportion to physical and radiographic findings. Rapid diagnosis is important and although PCP can be suggested by the presence of diffuse bilateral pulmonary infiltrates on radiographs and CT scans, diagnosis requires confirmation by direct detection of the organism in secretions. Direct staining of secretions from either induced sputum or bronchoalveolar lavage can be accomplished with immunoflourescent antibody stains and direct staining with Giemsa, Wright, and Gomori methenamine silver preparations. Treatment is with trimethoprim–sulfamethoxazole. Inhaled pentamidine has also been utilized and adjuvant corticosteroids may improve oxygenation in cases associated with significant hypoxia. Long-term prophylaxis in all intestinal patients is recommended. The ideal agent for prophylaxis is TMP–SMX, which is generally well tolerated and provides excellent prophylaxis against toxoplasmosis and *Nocardia*, both potentially fatal opportunistic infections in immunosuppressed patients [66–68].

15.6.3 Diarrheal Illnesses

Infectious diarrhea is a frequent complication after intestinal transplant and represents a frequent and clinically challenging differential diagnosis. Significant osmotic diarrhea in a patient with an intestinal allograft is rejection until proven otherwise. Fever is as likely to be a manifestation of intestinal rejection as infection and cannot be used to differentiate the two and the treatment of intestinal rejection, augmentation of immunosuppression, is the polar opposite of that for infectious diarrhea. Inappropriate use of immunosuppression to empirically treat rejection in a transplant patient can, and has been, a fatal mistake. Conversely, delayed treatment of severe rejection due to a suspected infectious cause can lead to exfoliative rejection and graft loss. Unfortunately, gross inspection of the allograft is nonspecific and requires histologic inspection to adequately differentiate between severe allograft enteritis and rejection. In addition, the ability to make the diagnosis is further confounded by the possibility that the two processes can occur simultaneously or that rejection can rapidly follow an episode of infectious enteritis, making serial endoscopic investigations necessary. In the end, the differentiation between infection and rejection relies on the balance of clinical suspicion, presentation, and direct inspection of the allograft. Rapid processing and rapid availability of biopsy samples is required to aid in the timely differentiation between the two entities.

Rotavirus. Historically, rotaviral enteritis (RVE) is a common cause of diarrhea in children and has been a significant cause of intestinal allograft enteritis, manifested as severe osmotic diarrhea without blood or abdominal pain. Fever is not generally a feature and the illness is generally self-limited, lasting roughly 10 days. Close follow-up is required for patients experiencing RVE of the intestinal allograft, as the postinfectious period is associated with a dramatically increased risk of cellular rejection, with 70 % of patients experiencing rejection, either concurrently or at a mean of 22 days after the RVE [69, 70].

Other Agents. The intestinal allograft can be affected by any of the other common infectious etiologies of diarrhea. Adenoviral enteritis was found in an additional 20 % of cases [70]. Interestingly, the most discussed agents, CMV and

EBV, were not frequent causes of diarrhea in intestinal recipients. *Clostridium difficile* is another common cause of infectious diarrhea after SOT (including intestinal transplant recipients) with a prevalence of 2.7 % in a recent report of patients undergoing SOT [71]. Although specific reports describing the course of CDI in intestinal transplant recipients are lacking, however it was found to be the etiology of diarrhea in 15 % of these patients and responded to standard antibiotic therapy [70]. Recurrent CDI has been shown to be associated with risk factors such as increased LOS, prolonged antibiotic courses, high levels of immunosuppression, and other comorbid conditions [71]. Parasitic enteritis is also seen n ITx recipients. Infection due to *Cryptosporidium* sp. has been reported in intestinal recipients [70] as has infection due to *Giardia lamblia* [70]. Recent attention has begun to focus on norovirus as a cause of enteritis in SOT recipients. Despite its self-limited course in the general population, norovirus has been demonstrated to be the cause of prolonged diarrhea with severe dehydration in pediatric intestinal transplant recipients and infection is associated with a prolonged viral shedding [72]. Diagnosis can be difficult, as intestinal epithelial apoptosis is a common finding in human calicivirus enteritis, making differentiation for rejection difficult. If clinically suspected, however the diagnosis can be confirmed with PCR analysis of biopsy specimens or effluent [73].

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Part III
Specific Sites of Infection

16 Pneumonia After Hematopoietic Stem Cell Transplantation

Catherine Cordonnier

Pneumonia is the most common infection after transplantation, and the infection with the highest mortality. Roughly two thirds of pneumonia observed after HSCT are of infectious origin, and this observation should be a priority leading the investigations. While the infection-related mortality has decreased after HSCT over time [1], it is not sure that the incidence of pneumonia decreased in parallel. Up to 30% of the patients may develop pulmonary symptoms within the first 100 days after allogeneic HSCT [2]. Even in T-cell-depleted allogeneic HSCT where the incidence of pneumonia seems to be low [3], the occurrence of pneumonia significantly impacts on survival. The rates of bacterial, viral, and polymicrobial pneumonia do not seem to be different during the first 3 months after transplant between allogeneic and autologous HSCT recipients, while the rate of invasive fungal disease (IFD) is much higher after allogeneic HSCT [2], due to a more severe and prolonged immune defect which also favors late infectious complications [4].

Factors enhancing the risk of infectious pneumonia are many and include donor and recipient serologies; previous pneumonia, which may warrant secondary prophylaxis; graft source; choice of donor and conditioning; graft-versus-host disease (GVHD); and also environmental factors. One of the main concerns in pneumonia evaluation is to distinguish infectious and noninfectious pneumonia since many noninfectious causes may mimic infection. Additionally, pulmonary coinfections are frequent. This makes that the results of indirect markers, even though extremely useful in practice, should be cautiously considered as it may identify only part of the responsible pathogens. Only a direct investigation of the lung as provided by bronchoalveolar lavage (BAL), combined with the use of well-chosen indirect markers, gives the best chances to identify several causes of pneumonia.

This chapter focuses on the factors that make the lungs particularly susceptible to infections after HSCT, the main specificities of clinical and imaging presentation of pulmonary infections, and the principles of diagnosis and management.

16.1 Altered Pulmonary Defense After HSCT

The lungs of HSCT candidates may have been exposed to toxic insults from their underlying diseases, prior infection, and prior chemotherapy and irradiation which may compromise normal surveillance barriers. Conditioning before transplant and subsequent immunosuppressive therapy and infection all may impair native defenses and increase the risk for pulmonary infection.

The ciliated and squamous epithelium, from nasopharynx to distal bronchioles, is the first line of defense. Significant impairment of the ciliary epithelium has been reported even years after transplant [5]. The respective role of viral or mycoplasma infection or of GVHD or radiation in this finding cannot be precisely determined. However, these abnormalities were found in 17 of 20 long-term allogeneic HSCT survivors and are probably underestimated in routine practice.

Alveolar macrophages act as phagocytes and secrete cytokines and chemokines providing a next level of defense. Their functions may be altered by immunosuppressive agents and viral infection. During prolonged neutropenic phases, the number of alveolar macrophages decreases, and this could favor infection from pathogens, which are normally phagocyted at the alveolar level [6]. Additionally, after allogeneic HSCT, the recipient alveolar macrophages are progressively replaced by cells of donor origin, and this may partly explain the numeric and functional impairment of the alveolar macrophage population during the first months after transplant [7, 8].

16.2 Evolution of the Problem

The occurrence of infectious pneumonia relates to the interrelationship of infectious exposure or reactivation, the condition of the lungs, and the degree of immunosuppression. The changes in many transplant procedures, including various

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prophylaxes, and the availability of new diagnostic tools over the last decade should have changed the incidence of pneumonia after HSCT. However, there is no clear data to support this hypothesis, and one may consider that these changes have more resulted in a change in timing and causes of pneumonia rather than in incidence or mortality. The increasing use of reduced intensity conditioning (RIC) regimens has significantly decreased the formerly high rate of early bacterial pneumonias. However, concomitantly, multidrug-resistant (MDR) bacteria have become a global concern in most hematology wards [9, 10]. The use of RICs has also changed the kinetics of many complications, delaying the onset of GVHD and the subsequent infections [11, 12]. Preemptive and prophylactic strategies of CMV infection have also considerably reduced the incidence of CMV pneumonia which nowadays affects less than 6% of the patients [13, 14]. However, pneumonia due to respiratory viruses has become common. New antifungal agents have improved therapeutic options for *Aspergillus* infection, but non-*Aspergillus* molds, especially mucormycoses, are being seen with increasing frequency [15–18]. Finally, despite significant progresses, the morbidity and mortality of pneumonia after HSCT remains one of the highest of any transplant.

The timing of infectious pneumonia follows the timing of other infections according to the type of transplant and occurrence and severity of GVHD which is the main factor prolonging the infectious risk after the neutropenic phase [4]. HSCT recipients are both at risk for nosocomial and community infections according to the phase of transplant. These environmental risks cannot always be prevented, on the contrary of the reactivation risks which must be evaluated before transplant.

16.3 Main Causes of Infectious Pneumonia After HSCT

Although changes in the transplant procedures have impacted on the infectious complications and their timing (see Chap. X), infectious pneumonia after HSCT occurs in predictable risk periods. After allogeneic transplant, early bacterial pneumonia mainly complicates myeloablative transplant, while opportunistic fungal and viral infections may affect the patient irrespectively of the type of conditioning. After autologous transplant, most pneumonias occur during the neutropenic phase, especially in myeloma patients [19], and few of them are of fungal origin [16].

16.3.1 *Bacterial Pneumonia*

Bacterial pneumonia occurring during the initial neutropenia are caused by pathogens common to all neutropenic patients or to those with comparable mucositis in the ward. The clinician should also consider the possibility of streptococcal pneumonia or ARDS related to streptococcal sepsis. These infections are particularly due to *Streptococcus viridans* and have been correlated with the presence of mucositis, the use of prophylactic quinolones, and the administration of high doses of cytarabine (see Chap. 20). The approach to bacterial pneumonias early after transplantation is similar to that in other neutropenic hosts, and it should include coverage for *Pseudomonas* species and eventually MDR in case of previous colonization or infection [20, 21].

Most patients are maintained on indwelling intravenous catheters throughout this period, and seeding of the lungs from bacteremia continues to be a potential risk. After recovery from neutropenia, allogeneic transplant recipients continue to be at risk for any nosocomial infections as long as they stay in the hospital (see Figure 16-1). Bacterial infections occurring in the late posttransplantation period may be favored by persistent immunoglobulin deficiency, which increases the risk of pneumonia caused by encapsulated bacteria.

Invasive pneumococcal infection occurs significantly more often after allogeneic, than after autologous, transplantations and especially in case of chronic GVHD [22–24]. They may be rapidly fatal. In a prospective study from the European Blood and Marrow Transplantation Group [22], no pneumonia developed in seven cases of invasive infection observed before day 100, whereas it was seen in 18 of 44 (41%) cases observed after day 100, and half of the fatal cases of late infection were associated with pneumonia. Early immunization with the 13-valent conjugate vaccine, completed by the 23-valent polysaccharide later, or a fourth dose of the conju-

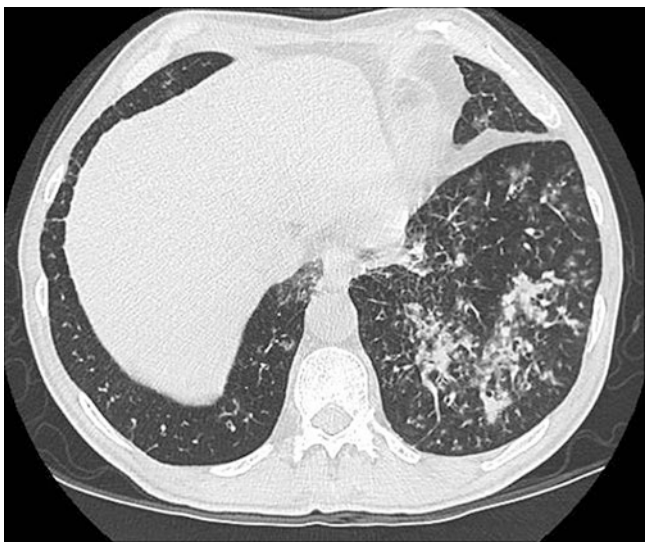

FIGURE 16-1. This 56-year-old patient has received an allogeneic HSCT from an unrelated donor for acute myeloid leukemia. He was smoker and suffered from chronic bronchitis before transplant. He was rehospitalized at 7 months after transplant for severe chronic GVHD and was treated with steroids. He developed febrile pneumonia after 9 days of hospitalization. The lung CT scan showed ground-glass, patchy infiltrates of the left lower lobe. The bronchoalveolar lavage was positive for coronavirus, and the culture of protected aspiration (10^3 CFUs/mL) and the culture of the lavage fluid (10^4 CFUs/mL) were both positive for *Klebsiella pneumoniae.*

gate vaccine in case of GVHD could reduce the incidence of pneumococcal infection over time [25, 26] (see Chap. 48). Similarly, *H. influenzae* may cause pneumonia and sinus infection, usually past the third month after transplantation. Immunization with a conjugate vaccine against type b is recommended from 6 months after transplant.

Pneumonias from *intracellular pathogens* are rarely reported, but they may recur in previously exposed patients. Pneumonia due to *Legionella* species has occasionally been reported in the setting of outbreaks, most often as a nosocomial infection. The radiologic findings may be variable; they may mimic fungal nodules, and they may not be apparent at the onset of high fever and pleuritic pain. Invasive nocardiosis, reported in 0.3–1.7 % after allogeneic transplant, mainly occurs in patients who are not receiving TMP-SMX and is often difficult to differentiate from fungal pneumonia [27, 28].

Mycobacterial infections due to *M. tuberculosis*, *Mycobacterium avium-intracellulare* complex, or other species are rarely reported. Generally, they are diagnosed at 2–18 months after transplantation, but they may develop early when prior infection has occurred (see Figure 16-2) [29, 30].

16.3.2 *Fungal Pneumonia (including pneumocystis pneumonia)*

Fungal pneumonia: *Aspergillus* is the most worrisome cause of IFD after allogeneic HSCT. It reportedly occurs after 0–20 % of transplantations; the most common site is the lung, and GVHD is the main risk factor (see Chap. X). A first peak of incidence occurs during the neutropenic period after myeloablative conditioning regimens, particularly in patients with leukemia. The second incidence peak is generally seen later in patients with acute GVHD and receiving corticosteroids. The availability of antifungal azoles for anti-aspergillus prophylaxis has significantly reduced the incidence [31–33]. However, the mortality of *Aspergillus* remained close to 50 % in recent series. This infection must be considered in any case of fever, particularly in that occurring in the patient on broad-spectrum antibiotics, or of any pneumonia, whether of new onset or a previously diagnosed condition that does not resolve with appropriate therapy (see Figure 16-3). A negative bronchoscopy result, even when combined with testing of galactomannan in the BAL fluid, does not diminish the suspicion for this pathogen. Without secondary prophylaxis eventually combined with surgical removal of the main lesions, the risk of relapse of prior *Aspergillus* infection after HSCT has been estimated around 20 % [34].

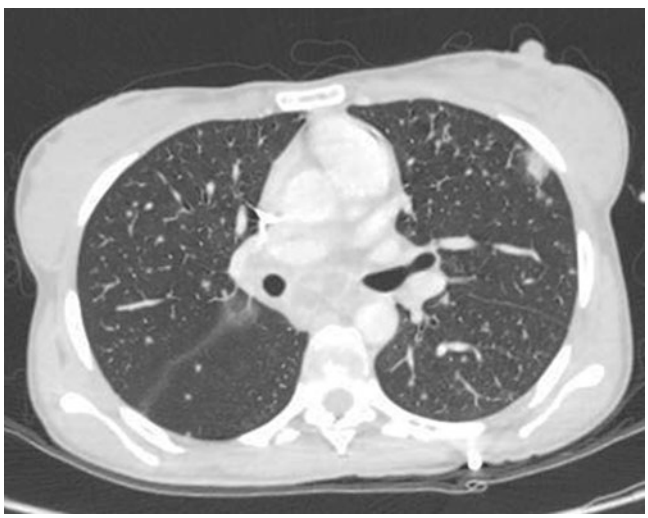

FIGURE 16-2. This 37-year-old woman received an allogeneic HSCT from her HLA-identical brother for poor-risk acute myeloid leukemia. She had a past history of pulmonary tuberculosis 10 years ago, but was intolerant to secondary prophylaxis. Three months after transplant, while she was well with no GVHD, she developed an insidious fever. Chest X-ray was normal. The lung CT scan showed diffuse micronodular infiltrates and a sub-parietal nodule of 1.5 cm in diameter in the upper left lobe. The bronchoalveolar lavage was positive for *M. tuberculosis* in culture.

In addition to being found in the lung parenchyma, *Aspergillus* may be isolated in the tracheobronchial tree where it may be responsible for significant airway obstruction. White, adherent plaques may be seen on bronchoscopy, particularly in the setting of chronic GVHD and steroid use. This infection must be differentiated from worsening bronchiolitis, so that inappropriate and dangerous increases in immunosuppression can be avoided.

Pneumonia due to *Candida* species is rarely reported, partly because no firm criteria for differentiating invasive infection from colonization based on bronchoscopy without biopsy exist. The lungs may be involved in any systemic *Candida* species infection.

Pneumonias due to endemic fungi, such as *Histoplasma* or *Coccidioides* species, particularly in North America, must be considered in these patients, as should the emerging fungi, including *Trichosporon*, *Alternaria*, and *Fusarium* [16].

A special attention should be paid to the possibility of *Mucorales* after allogeneic HSCT (see Chap. 39). Its mortality rate is between 50 and 80 % [18, 35–37]. Mucormycosis shares with aspergillosis common risk factors but usually occurs later, and often after voriconazole administration, although the role of a selection pressure is debated [35]. There is no indirect available marker of mucormycosis except PCR test currently in evaluation [38]. The classical presentation of mucormycosis after transplant mostly mimics aspergillosis, but galactomannan is negative (see Figure 16-4). Differentiating mucor from aspergillus infection is, however, of great importance due to different therapeutic implications. As long as there is a doubt between the two infections, the patient must be treated with liposomal amphotericin B.

Pneumocystis jirovecii Pneumonia (PjP) Historically, the incidence of PjP in patients not receiving prophylaxis in the 1980s was found to be 16 % during the first 6 months after transplant [39, 40]. This incidence has dramatically decreased between 1 and 2.5 % [41, 42] with the use of trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis, but the mortality in established PjP remains around 50–70 % [43–45].

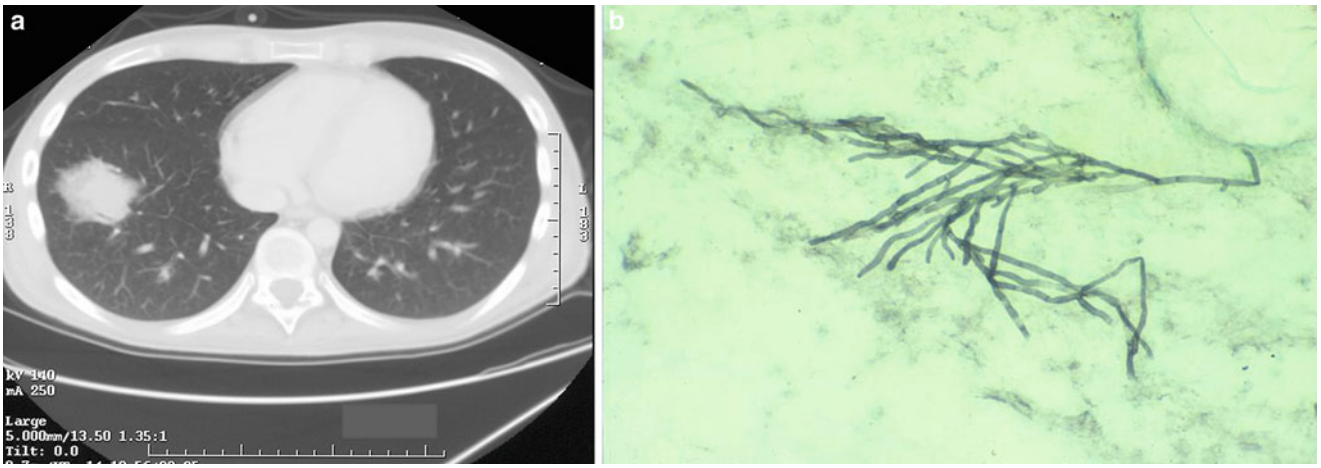

FIGURE 16-3. This young patient, 20 years old, received an allogeneic HSCT from an unrelated donor for acute lymphoblastic leukemia in second remission. He got severe acute GVHD and was not compliant to anti-mold azole prophylaxis. He developed an acute right chest pain with fever. (**a**) Both X-ray and CT scan showed a macronodular and isolated lesion of the right lower lobe. Serum galactomannan assay was negative. (**b**) The bronchoalveolar lavage smears showed hyphae characteristics of aspergillus (Gomori-Grocott stain). The culture of BAL fluid grew to *Aspergillus fumigatus.*

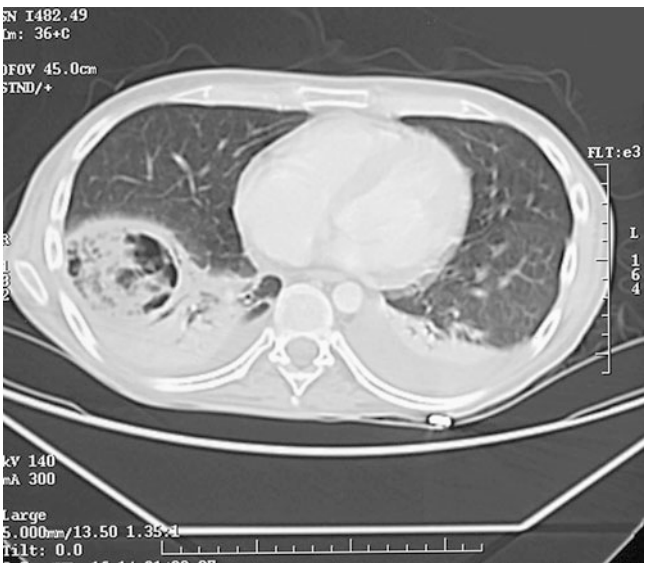

FIGURE 16-4. This 28-year-old patient had received an allogeneic HSCT for acute lymphoblastic leukemia from an unrelated donor. He got severe, cutaneous, and gut GVHD and was treated with steroids. At 4 months after transplant, while still on 0.7 mg/kg of prednisone, he developed a nodular lesion of the right lower lobe. A galactomannan test was positive in serum. He refused fibroscopy and was treated for aspergillus infection with voriconazole. He then did not attend the consultations for 1 month and came back with bilateral thoracic pains and fever. The CT scan showed bilateral pleural effusion and a voluminous round, necrotic lesion surrounded by an area of consolidation in the right lower lobe. Rhizopus grew from the BAL fluid.

However, in patients receiving dapsone prophylaxis, an incidence of 7.2 % was reported after allogeneic HSCT [43]. PjP usually manifests with fever, nonproductive cough, dyspnea, and diffuse interstitial pneumonitis. In HSCT recipients, the presentation of PjP may be extremely abrupt, and the patient may quickly deteriorate and require intensive care unit (ICU) [46–48]. Rarely, the disease may reveal by an isolated low-grade fever and a normal chest X-ray at the beginning. In such cases, if the cause of fever is not rapidly found, a CT scan will show pulmonary ground-glass lesions and prompt a BAL [49]. The elevation of LDH is poorly helpful [46]. Most patients present with nodular infiltrates or other pattern of diffuse interstitial pneumonia. Pleural effusion and pneumothorax are uncommon [44]. Most cases occur between 3 and 24 months after transplant, in patients with acute or chronic GVHD or in relapse of the underlying disease [42, 43, 49, 50]. Most are receiving steroids, especially at a phase of tapering off, or after recent withdrawal, and do not receive, or are not compliant to, TMP-SMX prophylaxis [51]. Whether a low CD4 count is a main risk factor for developing PjP after HSCT is unknown.

P. jirovecii is not cultivable in vitro. It may be identified by microscopic detection, direct or indirect immunofluorescence (IF), or nucleic acid tests (NAT) (see Figure 16-5). Several stainings may be used for microscopic detection of trophic forms and cysts in any respiratory sample such as Giemsa to identify trophic forms and toluidine blue O or calcofluor white to detect cysts, without significant difference in their diagnostic performance. IF has a better sensitivity than conventional stainings [52, 53]. The combination of one classical staining and IF allows the detection of both cystic and trophic forms. PCR is the most sensitive diagnostic assay to identify pneumocystis [54–56], although no study defines a clear cutoff of positivity [57, 58].

HSCT recipients, as other non-HIV-infected patients, are known to be infected with low burden of cysts [53, 59, 60]. As there is a decreasing gradient of the pneumocystis burden from upper to lower respiratory airways, this probably explains

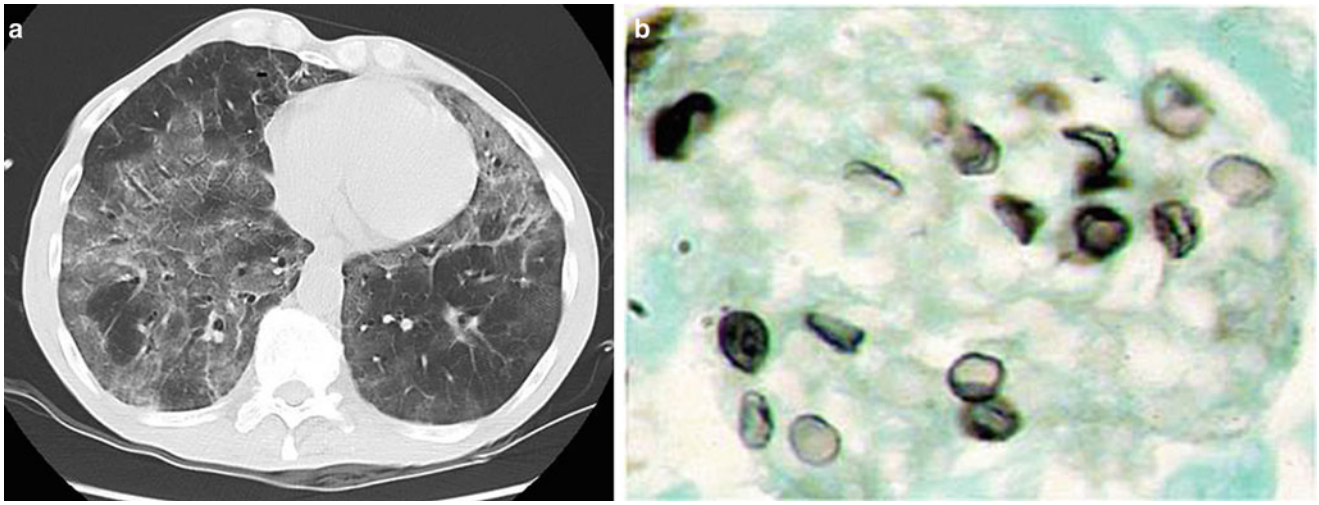

FIGURE 16-5. This 62-year-old patient received an autologous peripheral blood stem cell transplantation for non-Hodgkin lymphoma. He developed a severe rash under trimethoprim-sulfamethoxazole at the end of the first month after transplant. He was therefore switched to atovaquone for *P. jirovecii* prophylaxis. Five months after transplant, he developed fever and rapid respiratory failure with hypoxemia. A chest X-ray showed slight interstitial, bilateral lesions. (**a**) The CT scan showed bilateral ground-glass lesions predominant on lower lobes. (**b**) The bronchoalveolar lavage showed characteristic cysts of *P. jirovecii* on Grocott staining. IF and qPCR were also positive.

the difficulties to identify *P. jirovecii* in induced sputum or other upper respiratory samples with conventional techniques in non-HIV-infected patients. Therefore, BAL fluid is the preferred specimen for the diagnosis of PjP in HSCT recipients. Another argument for BAL is that half of the PjP cases in non-HIV-infected patients are associated with coinfections, especially with bacteria, CMV, and *Aspergillus spp*. [44, 46, 61] which require identification and treatment.

In case a BAL cannot be done, upper respiratory tract (URT) specimens, like induced sputum, oral washings, nasal swabs, or nasopharyngeal aspirates, can be used, but with a lower expected diagnostic value than with BAL. Serum (1-3) β(beta)-D-glucan is a major cell wall component of *P. jirovecii*. Two meta-analyses [62, 63] have shown its excellent sensitivity, but due to its panfungal nature and the frequency of other IFD after HSCT, it can be only a screening tool for PjP. On the other hand, its use in BAL fluid is not recommended, due to a poor sensitivity and reproducibility [64, 65]. The recent guidelines of the fifth European Conference on Infections in Leukemia [66] propose a practical algorithm for the diagnostic of PjP in non-HIV-infected patients, based on the examination of BAL fluid with IF and qPCR. The positivity or negativity of both techniques signs the presence or absence of PjP. When IF is positive, and qPCR negative, this should reflect a technical problem, mainly of qPCR. When qPCR is the only positive assay, although no quantitative cutoff can be uniformly proposed, a high fungal burden favors a diagnosis of PjP. The concomitant positivity of serum (1-3) β(beta)-D-glucan is an additional argument favoring PjP. When BAL is not possible because the patient is too hypoxemic or refuses the procedure, serum (1-3) β(beta)-D-glucan can be helpful in conjunction with URT samples. When the clinical suspicion of PjP is high and the BAL cannot be done immediately, an empirical treatment with TMP-SMX should be started as soon as possible since it will not impair the diagnostic yield of investigative procedures before at least several days. TMP-SMX at the dose of 15–20 mg/kg of TMP plus 75–100 mg/kg of SMX, by oral or preferably IV route, is the first choice for treatment [67], even in patients who were supposed to take TMP-SMX prophylaxis as the presence of dihydropteroate synthase mutations does not significantly affect the treatment efficacy [68]. The addition of steroids for the more hypoxemic patients (PaO2 while breathing room air <70 mmHg), although well established in HIV-infected patients [69], is debated in others.

PjP prophylaxis is strongly recommended from engraftment for at least 6 months after allogeneic HSCT and longer as far as any immunosuppressive drugs are administered [70, 71] and for at least 3–6 months after autologous HSCT [70]. No large prospective series compare the respective prophylactic efficacy of TMP-SMX with alternatives in HSCT recipients. However, strong arguments from both acquired immunodeficiency syndrome prospective studies and HSCT retrospective series suggest that TMP-SMX is the best prophylactic regimen [43, 72], any alternative to TMP-SMX—dapsone, atovaquone, or pentamidine—being inferior [71].

16.3.3 Viral Pneumonia

During the neutropenic phase of transplant, the incidence of herpes simplex virus (HSV) reactivation and disease—including pneumonia—has fallen sharply with the wide use of prophylactic acyclovir or valaciclovir [73].

Until the beginning of the 1990s, CMV was the most significant pathogen for pneumonia after allogeneic transplant, affecting 15 % of the recipients. Preemptive and prophylactic strategies have greatly decreased its incidence, currently in the range of 1–5 % [14, 74–76]. It is generally a febrile disease in which the radiographic patterns are primarily interstitial but sometimes alveolar. Coinfections are frequent. The optimal approach to identify the virus in the lungs is the combination of IF and rapid culture of BAL fluid. The identification of CMV through PCR on BAL fluid has been shown to have limited correlation with the development of CMV pneumonia and therefore is not considered as criteria for CMV pneumonia [77] (see Chap. 24). Therefore, as most of the laboratories abandon IF assays to more automated qPCR techniques, a careful examination of the BAL smears by an experimented cytologist is important to detect the cytological hallmarks of CMV pneumonia, knowing that the identification of the characteristic inclusions in alveolar cells is a sign of advanced infection [78] (see Figure 16-6).

Other herpesviruses, including varicella-zoster virus, EBV, and *Human herpesvirus* 6 (HHV-6), have been reported as causes of pneumonia in HSCT recipients. High levels of HHV-6 DNA have been found in the lung tissue of patients with idiopathic or CMV interstitial pneumonitis [76]. However, the clinical significance of this finding, and the need for specific therapy, is still unclear.

Pneumonia caused by respiratory viruses has become a main concern in HSCT recipients. The list regularly enlarges [79, 80]. The main risk factors for death are the early onset after transplant, neutropenia, lymphopenia, GVHD, steroid administration, and older age [79, 81–83]. Recently, an immunodeficiency scoring system has been proposed to predict poor outcomes and better identify patients infected by respiratory syncytial virus and who should benefit the most from antiviral therapy [83]. The incidence is lower after autologous than after allogeneic transplant [84]. Identification by NAT in respiratory samples is the recommended technique and may be performed on nasopharyngeal or throat swabs, bronchial aspiration, or BAL fluid [79, 85, 86] with multiplex assays. Diagnosing these patients early has several benefits: [1] some of these infections may be efficiently treated (e.g., oseltamivir in influenza infection or ribavirin for respiratory syncytial virus); [2] all of them imply isolation and barrier measures to prevent transmission to other patients or staff; [3] respiratory viral infections early after allogeneic transplant predict the development of alloimmune lung syndrome, including bronchiolitis obliterans and idiopathic interstitial pneumonia [79, 87, 88]. When respiratory viruses are detected before transplant, delaying the transplant should be considered [89].

Measles pneumonia has rarely been reported after HSCT but may be an expected event in the setting of outbreaks [90] and may occur without a rash. Adenovirus pneumonia is a very rare but potentially life-threatening event occurring in the setting either of disseminated adenovirus infection or of usually upper and then lower respiratory tract infections [91] (see Chap. 33) and occur more frequently in children than in adults and in unrelated transplants or after T-cell depletion.

16.3.4 Other Causes

Reports of pulmonary *toxoplasmosis* are rare; it is usually seen in the setting of disseminated infection resulting from reactivation, during the first year after transplantation in seropositive recipients not receiving TMP-SMX. The pattern is usually a diffuse interstitial disease, and neurologic symptoms may be absent. *Toxoplasmosis* may be identified in BAL fluid and blood by IF and qPCR. A prospective screening by qPCR in the patients at risk may allow a preemptive therapy [92].

16.4 Differential Diagnosis to Infectious Pneumonia: The Main Noninfectious Processes Affecting the Lungs After HSCT

The lung is the site of numerous noninfectious injuries causing one third of pulmonary infiltrates after HSCT. This needs to be considered because they may require specific treatments. Pulmonary edema, pulmonary embolism, and acute respiratory distress syndrome may occur at any time, but more often during the early phase of transplant, without any special presentation in transplant recipients and will not be detailed here. Other noninfectious processes affecting the lung deserve specific consideration as they are either frequent or specifically observed in HSCT recipients. These noninfectious processes may be associated with infections, increasing the difficulty to propose optimal treatment. The best identification is however of crucial importance since steroids may be indicated in several noninfectious processes while they will be deleterious in most infections. The probability of their occurrence may vary by time after transplantation and type of transplant.

Alveolar hemorrhage (AH) is a frequent noninfectious process affecting the lung after any HSCT, with an incidence rate of 6–41 % [93–95]. AH is diagnosed on the basis of either a bloody aspect of the BAL fluid—usually transient—or the presence of ≥20 % of siderophages among alveolar macrophages (see Figure 16-7) [96]. AH after HSCT may be an autonomous process favored by thrombocytopenia, other coagulation disorders, or renal failure [96] and by any rupture of the alveolar-capillary barrier such as in pulmonary edema, but it may also be associated with infections, like aspergillus or CMV, in two thirds of the cases [94, 97].

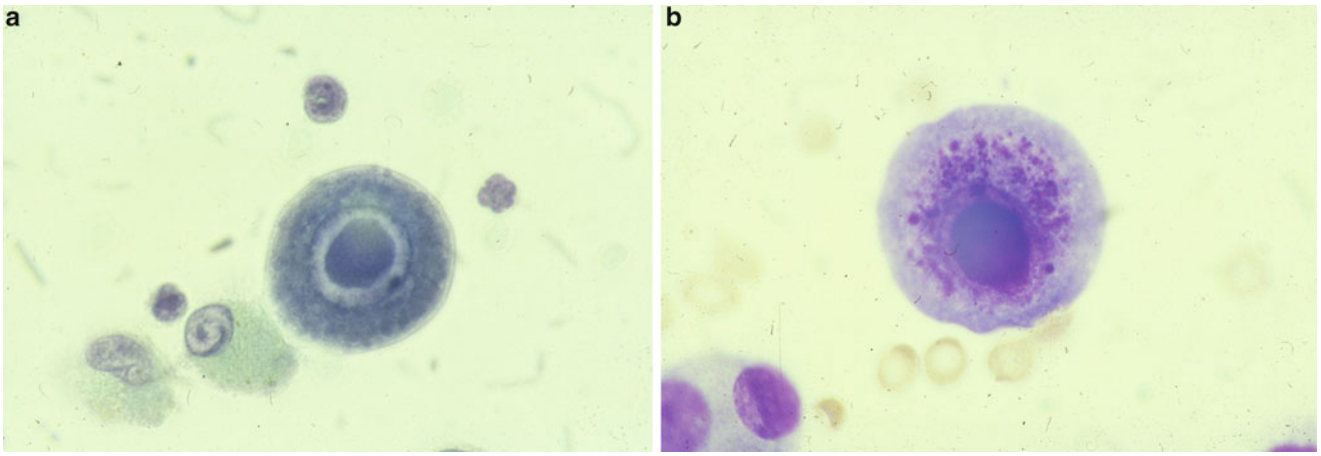

FIGURE 16-6. Bronchoalveolar cytocentrifuged smears in CMV pneumonia. (**a**) Papanicolaou staining: The slide shows the cytomegaly of an infected alveolar macrophage and, additionally, typical haloed "owl-eyed" basophilic intranuclear inclusions (Papanicolaou staining). (**b**) May-Grunwald-Giemsa staining: Intracytoplasmic inclusions, which are pathognomonic of CMV infection.

Neither clinical presentation nor imaging are specific of infectious or noninfectious forms [97].

Secondary alveolar proteinosis (**AP**) is rare, occurring mostly during prolonged neutropenia. It is the result of a complex process probably combining pneumocyte II stimulation and quantitative and functional defects of the alveolar macrophages. This results in an impaired clearance of pulmonary surfactant and the accumulation of a lipoproteinaceous periodic acid-Schiff (PAS)-positive material in the alveolar space (see Figure 16-8) [98, 99]. It usually mimics an insidious pulmonary edema. The diagnosis may be suspected on the sticky aspect of the BAL fluid and then by difficulties to count the cells. The usual stainings do not identify AP. The cytologist must be aware of this possibility and examine the alveolar material on PAS or Black Sudan staining. Secondary AP rarely complicates with severe respiratory failure [99]. When it occurred during neutropenia, it usually improves at neutrophil recovery. However, as for AH, some cases are associated with infections.

Pulmonary veno-occlusive disease is a very rare event after HSCT. It mainly manifests by pulmonary arterial hypertension, but with a normal pulmonary artery occlusion pressure. The diagnosis is extremely difficult. By analogy with liver veno-occlusive disease, it is hypothesized that it is due to chemotherapy and/or radiation toxicity on the small vessels [100, 101].

The engraftment syndrome may be observed during neutrophil recovery, at a median onset of 16 days after transplant, and usually associates ≥2 of the following criteria: fever, skin rash, weight gain due to capillary leakage, and respiratory failure without other identified cause [102]. It is hypothesized that degranulation of upcoming neutrophils could induce lung injury. Engraftment syndrome is associated with a large dose of mononuclear cells infused, the use of G-CSF or GM-CSF, early neutrophil recovery, non-myeloablative conditioning, the use of amphotericin B therapy, and autologous rather than allogeneic transplant [102–105]. An incidence up to 48 % has been reported in children after allogeneic myeloablative transplant, one fourth of them suffering from pulmonary symptoms. As severe patients may require steroids [104, 105], it is important to quickly rule out an infection.

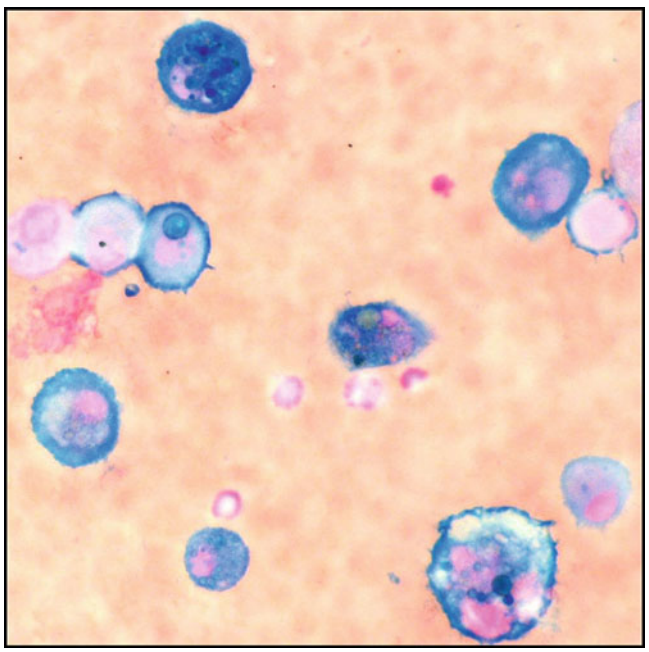

FIGURE 16-7. Alveolar hemorrhage: Bronchoalveolar lavage cytocentrifuged slide stained by Perls' Prussian blue method. The hemosiderin-laden macrophages (siderophages) characteristic of alveolar hemorrhage are identified by their blue cytoplasm (Courtesy of Dr. Jeanne Tran Van Hieu, Pathology department, Henri Mondor University Hospital, Créteil, France).

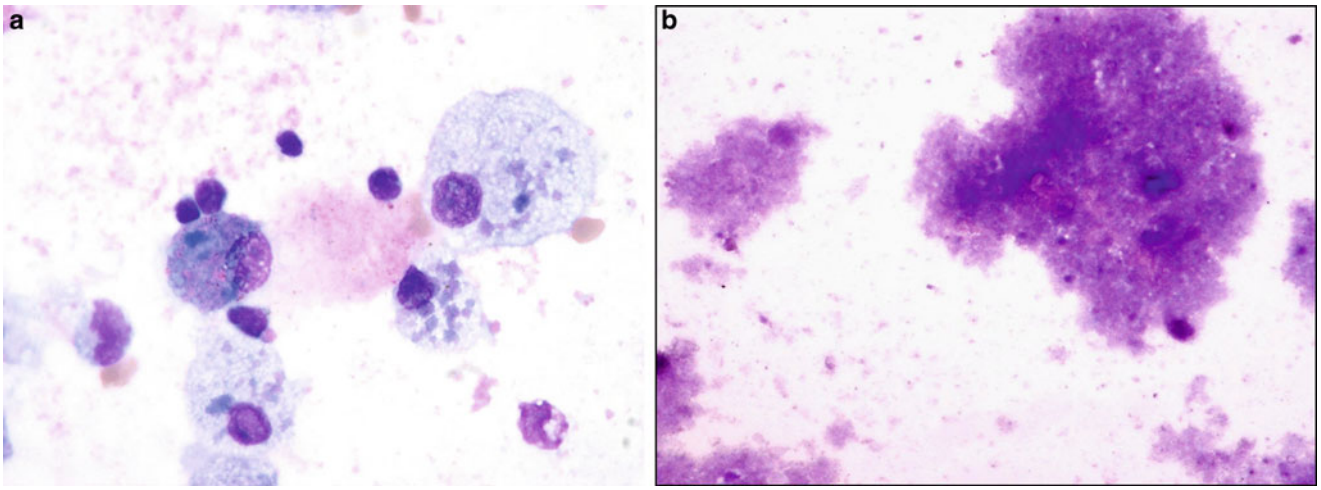

FIGURE 16-8. Secondary alveolar proteinosis: Bronchoalveolar lavage, May-Grünwald-Giemsa (MGG) staining. The presence of eosinophilic pale floccular material between alveolar macrophages and inflammatory cells on MGG-stained slides is highly suggestive of lipoproteinous material (**a**). This can be confirmed using the periodic acid-Schiff (PAS) method that showed positive staining (**b**) (Courtesy of Dr. Jeanne Tran Van Hieu, Pathology department, Henri Mondor University Hospital, Créteil, France).

Idiopathic (noninfectious) interstitial pneumonia is a complication reported in most allogeneic HSCT studies, with a high mortality rate. This diagnosis implies to have ruled out at least the main infections classically presenting as diffuse interstitial pneumonia, especially viral pneumonia and PjP, cardiac dysfunction, and fluid overload [106]. In myeloablative transplant, it has been associated with leukemia or myelodysplastic syndrome, severe acute and chronic GVHD, high-dose total body irradiation, and older age. In allogeneic HSCT, its incidence has been reduced from 8.4 % after myeloablative to 2.2 % after non-myeloablative conditioning [107]. A recent study showed that among 69 HSCT recipients who had developed an idiopathic pulmonary syndrome between 1992 and 2006 in Seattle, a retrospective microbiological screening of BAL material for 3 bacteria, 25 viruses searched with NAT, and galactomannan identified that 56.5 % of the patients had one pathogen (mainly HHV-6, rhinovirus, CMV, and aspergillus), and this finding was associated with an increased mortality at day 100 [76]. This confirms that the rate of "idiopathic" pneumonia is highly depending on how far infection is searched.

Bronchiolitis obliterans (BO or obliterative bronchiolitis) is an important factor contributing to death usually from 6 months after HSCT. Reported only after allogeneic HSCT, the condition has been related to older age, unrelated donor, total body irradiation, decreases in serum immunoglobulin G, and chronic GVHD, with a frequency of 3–10 % in patients with chronic GVHD who survive 120 days [108]. It seems to be prevented by T-cell depletion of the graft [109]. BO usually occurs insidiously, with cough, dyspnea, and wheezing, but may complicate with fever and mimic bronchopulmonary infection. Its hallmark is airway obstruction. The lung CT scan shows hyperinflated bronchiectasis, with a mosaic pattern. BAL and other endoscopic samples are of limited value as they just aim to rule out infection. As no noncontributory BAL can definitely rule out infection, it is preferable to perform two consecutive BALs at 1–2 weeks interval to increase the chance to not miss any pathogen. It is often associated with sinusitis and complicated by infections, especially those caused by *Haemophilus influenzae*, *S. pneumoniae*, *Aspergillus* species, and respiratory viruses. Despite immunosuppressors, the prognosis is poor.

Alveolar or nodular infiltrates may be seen in the setting of allogeneic HSCT as a result of **bronchiolitis obliterans organizing pneumonia** (BOOP)—also called cryptogenic organizing pneumonia [108, 110]. BOOP is much less common than BO and is also considered a manifestation of GVHD but has also been reported after autologous HSCT. It occurs earlier than BO, usually in the first 3 months following transplant. The CT scan shows nodular opacities and patchy consolidations. Pulmonary function tests show a restrictive defect. A histologic diagnosis is strongly recommended because BOOP may mimic infection, but can be reversible with corticosteroid therapy.

Malignant lung lesions may be seen after HSCT, either due to a primary or secondary cancer, localized relapse of the hematologic malignancy (see Figure 16-9), or EBV lymphoproliferative diseases (see Chap. X).

16.5 Principles of Management

Management of pneumonia after HSCT requires a high degree of suspicion and the early use of diagnostic procedures. The increasing availability of indirect markers of infection tends to decrease the early use of BAL. However, BAL remains the easier and safer procedure to identify both infectious and noninfectious causes of pneumonia. More invasive diagnostic

procedures such as transbronchial or lung biopsy need to be selected in situations in which BAL is noncontributory while weighing the risk of increased morbidity.

16.5.1 Clinical Approach to Pneumonia

A systematic approach to pneumonia in any HSCT recipient should include consideration of the following: history, clinical presentation, and imaging.

16.5.1.1 History

Knowledge of a patient's exposure, travel, environmental risks, and previous documented infection, the hospital epidemiology, and the pretransplant donor and recipient serologies particularly with regard to CMV and toxoplasmosis are essential. A history of recurrent MDR bacterial infection may require special consideration in choosing antibiotics [21]. Evaluation of the patient's compliance to anti-infective prophylaxis, especially to TMP-SMX, may be essential in evaluating the risk of PjP [51]. Whether the patient is neutropenic, lymphopenic, or hypogammaglobulinemic at presentation may be important to list the main infectious hypotheses.

16.5.1.2 Clinical Presentation

Symptoms and signs of pneumonia may or may not be typical of a known infectious cause. However, none is very specific. As in all immunosuppressed patients, few findings may be present, so any symptoms must be carefully and quickly evaluated, because of the consideration that any infection can rapidly progress. Fever, cough, or sputum production may be absent. Hypoxemia may be the sole finding, and even if the X-ray is normal, in case a chest CT scan cannot be obtained quickly, a bronchoscopic evaluation should be considered. The presence of any such symptom may, however, reflect a noninfectious etiology. Acute thoracic pain, with or without hemoptysis, may indicate embolic disease but may also denote *Aspergillus* infection. Pneumothorax may reveal—or complicate—PjP, mycobacterial or *Aspergillus* infection, or fibrosis. The rapid onset of pneumonia is mainly consistent with bacterial pneumonia, PjP, pulmonary edema or hemorrhage, or thromboembolism, but this may also occur with viral infections in immunosuppressed patients. A subacute onset more suggests IFD, although it may present abruptly.

16.5.1.3 Imaging

Posttransplantation pneumonia may be focal, multifocal, diffuse and interstitial, alveolar, or mixed. Every effort must be made to quickly obtain chest X-rays of optimal quality and/or a high-resolution chest CT scan when easily available. X-rays in supine position are rarely helpful. Additionally, most X-ray patterns are nonspecific and many patients have mixed types of infiltrates. When an X-ray appears negative or shows only minimal changes, there is good evidence that a chest CT may reveal abnormalities. CT scan has the best negative predictive value to rule out pneumonia and will show lung images 5 days before chest X-ray [111]. CT may additionally provide localization of the lesions, guiding invasive procedures, and inform on their proximity to pulmonary vessels. This information is also important to evaluate the

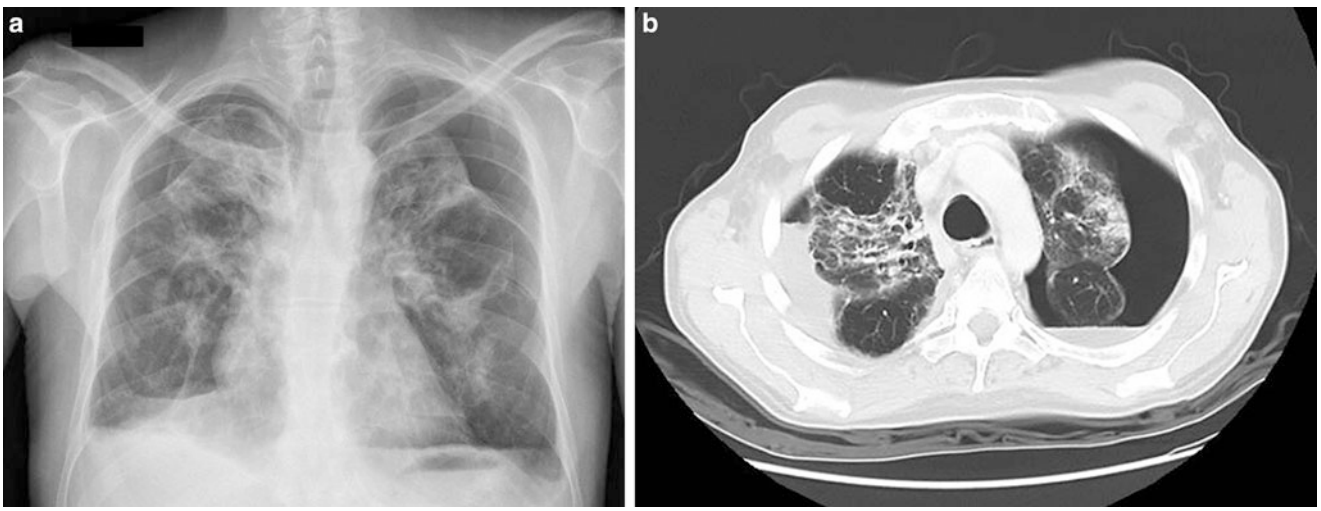

FIGURE 16-9. This 37-year-old patient received an allogeneic HSCT from his HLA-identical sister for refractory Hodgkin disease 20 months ago. He developed chronic respiratory failure due to concomitant causes: Hodgkin pulmonary relapse documented at 18 months and pulmonary fibrosis likely favored by previous mediastinum irradiation. (**a**) The chest X-ray shows bilateral partial pneumothorax, more important on the left side, bilateral pleural effusions, and multiple condensations. (**b**) The CT scan confirms the multiple retractile lesions of the lungs with bronchial dilatations and pleural thickening. It also confirms the left pneumothorax.

risk of hemoptysis in aspergillosis. CT may also detect small pleural effusions. Some CT findings may suggest the presence of particular infections. For example, the halo sign—a macronodule (≥1 cm in diameter) surrounded by a perimeter of ground-glass opacity—is very evocative of early aspergillosis during neutropenia [112], but may also be seen in other infections (e.g., legionella, mycobacterial infection, mucormycosis, or viral infections). Similarly, the reversed halo sign or "atoll sign"—a focal ground-glass attenuation surrounded by a ring of consolidation—has been shown to be often due to mucormycosis in hematology patients, but may also be observed in other infections, including aspergillosis [113]. Ground-glass opacities are very nonspecific and consistent with any infectious and many noninfectious processes such as pulmonary edema or hemorrhage. However, even with more characteristic lesions—such as the air crescent sign which is rare after HSCT but very evocative of mold infection—a CT scan does not replace the need for identification of the pathogen for diagnosis. Magnetic resonance imaging (MRI) usually does not provide more information than CT, except in the detection of lung abscesses [114]. The usefulness of PET scan is limited for diagnosis of acute pneumonia but may be better in nodular, subacute lesions [115], to identify extrapulmonary lesions or to follow the treatment efficacy [116–118]. Any workup using imaging should be completed rapidly, and it should lead quickly to a diagnostic procedure or, in most cases, to an empiric approach considering the most likely hypotheses.

16.5.2 Diagnostic Investigation

Blood cultures should be performed routinely, but they are of limited value in diagnosing pneumonia except for when the pathogen has a high propensity for the blood, such as *Streptococcus pneumoniae*, or in neutropenia. Special culture media are required when *Nocardia* or atypical mycobacteria are suspected. The blood should also be quickly sampled for CMV antigenemia or quantitative real-time PCR (qPCR) in patients at risk. The microbial documentation of any other site of infection, such as skin biopsy of cerebrospinal fluid, may be useful.

Blood biomarkers for the diagnosis of IFD include the detection of galactomannan by an enzyme-linked immunosorbent assay and of (1-3) β(beta)-D-glucan by a colorimetric assay. (1-3) β(beta)-D-glucan is a panfungal marker, while galactomannan is mainly associated with aspergillosis, although it may be positive in other mold infections, e.g., fusariosis. A meta-analysis of 27 studies showed that the galactomannan test has a sensitivity of 0.71 and a specificity of 0.89 for proven invasive aspergillosis [119]. The assay seems to be more useful for the prospective screening of neutropenic patients rather than for diagnosing pneumonia and also more useful in neutropenic than in non-neutropenic patients [120, 121]. The cutoff of positivity usually recommended is an index ≥0.5 in plasma or serum [121]. In an autopsy-based study, the sensitivity and specificity of the serum (1-3) β(beta)-D-glucan test for the detection of IFD were 95.1 % and 85.7 %, respectively [122]. Serum (1-3) β(beta)-D-glucan test is also very useful in the indirect diagnosis of PjP [63, 123]. Fungal NAT have also been widely investigated in HSCT recipient [124], but no consensus on their use in clinical practice currently exists. At this time, no noninvasive test that can replace the specificity of direct pulmonary investigation exists.

Although *sputum* may be analyzed to yield organisms colonizing the oropharynx, the clinical relevance of the results is not evidence based in the setting of HSCT. A positive culture may be valuable when agents that do not normally inhabit the oropharynx are isolated, especially *Legionella*, mycobacteria, and some fungi, or to document MDR colonization which may guide an empirical antibacterial treatment. In HSCT recipients with pneumonia, a positive sputum culture may be highly suspicious for pulmonary aspergillosis. Similarly, the presence of *M. tuberculosis* in the sputum may be considered the cause of the pneumonia when clinical and radiologic signs support this etiology. This assertion is to be considered with more caution for nontuberculous mycobacteria [125].

Nasopharyngeal aspirates or washings are useful to detect respiratory viruses in patients with URT infection [81, 84]. However, the correlation with the cause of the concomitant pneumonia is only presumptive as coinfections are frequent [84].

The standard for diagnosing pulmonary infection after HSCT is *bronchoscopic sampling with BAL* [126] (Table 16-1). Lavage is safe, minimally invasive, and reproducible. Its overall diagnostic yield is comparable to the one of lung biopsy, but with more infectious diagnostic and much less complications [126].

The clinician who consults with a pulmonary specialist for BAL should consider platelet transfusions if the patient is thrombocytopenic and should alert the microbiology laboratories to ensure that all potential organisms are sought. Oxygen saturation or arterial pressure should be assessed before the procedure. Fever, transient hypoxemia, and worsening of chest X-rays may be expected in as many as one half of patients during the few hours following the procedure [127]. When the patient is hypoxemic (paO2 < 70 mmHg spontaneously or with O2 supplementation) or tachypneic before BAL, he usually benefits from noninvasive ventilation immediately after the procedure. The overall diagnostic yield of BAL in infectious pneumonia occurring in hematologic patients varies between 27 and 55 % [2, 95, 128–131] depending on many parameters such as the following:

- The localization of the pulmonary lesions: whether they are accessible by BAL or not.

Table 16-1. Investigations on bronchoscopic samples in HSCT recipients

	Laboratory investigations	
Sample	Essential	Optional
Protected bacteriologic sample (brush or catheter)	Gram stain	Search for bacteria in neutrophils
	Quantitative cultures	
Aspiration	*Legionella*: immunofluorescence (IF), culture on BCYE medium or more selective media	India ink
	Mycobacteria and *Nocardia*: AFB stain, culture	
	Fungi: wet mount, culture	
Lavage fluid	Cytologic examination of lavage fluid on smear and after cytocentrifugation: direct examination, differential count, viral inclusions, pathogens	
	Stains	Stains
	–May-Grünwald-Giemsa	–Gomori methenamine silver (or alternative stain for *P. jirovecii*)
		–Papanicolaou
		–Periodic acid-Schiff
		–Perls' Prussian blue (hemosiderin-laden macrophages)
	Microbiologic processing	
	–Gram stain, bacterial culture	–Quantitative culture of BAL fluid
	–Legionella: culture on BCYE medium or more specific media	–PCR for *Legionella pneumophila*
	–Mycobacteria	–PCR for *Chlamydia pneumoniae*
	–Fungi: wet mount stain, culture	–PCR for *Mycoplasma pneumonia*
	–*P. jirovecii*: IF and/or qPCR	–PCR for *Mycobacterium tuberculosis*
		–Galactomannan antigen
	Virus	
	–All possible viruses, particularly the herpes family, adenovirus, and respiratory viruses: IF	–PCR for HSV, VZV, CMV, EBV, HHV-6
		–PCR for respiratory viruses and adenovirus
	Other	–Toxoplasmosis: IF, PCR
Transbronchial biopsy[a]	Histology	

[a]Transbronchial biopsy is essential for noninfectious processes and less contributive than BAL for infectious pneumonia. However, it is usually not proposed in the initial investigation of pneumonia, due to its possible complications (pneumothorax, bleeding).

BCYE buffered charcoal yeast extract, *AFB* acid-fast bacillus, *IF* immunofluorescence, *PCR* polymerase chain reaction.

- Whether the patient is neutropenic. The yield of the procedure is usually lower in neutropenic than in non-neutropenic patients [131].
- The type of the causal infection: for example, the diagnostic yield of BAL with conventional mycological techniques—without galactomannan tested in the BAL fluid—for aspergillus pneumonia is usually lower than 50 %, while it is higher than 90 % in PjP or CMV pneumonia, for which one rarely needs a lung biopsy [67].
- The laboratory exams performed on fibroscopic samples. The laboratory protocol should be established in advance in a multidisciplinary approach according to the expected, infectious and noninfectious, causes of pneumonia, eventually adapted to seasons for respiratory viruses.
- The criteria used to define specific entities. For example, it is generally believed that the presence of candida in a BAL fluid or bronchial aspiration does not necessary mean a candida pneumonia, while the presence of aspergillus in an HSCT recipient does [132]. However, for some causes of pneumonia, there are until now no consensus definition. The increasing availability of NAT for many pathogens should not replace, in many instances, more classical techniques, until the need for classical techniques is shown to be no longer useful in diagnosing a given infection.
- The delay elapsed between presentation and BAL and the number and duration of previous antibiotics before performing BAL [133]. The diagnostic yield of BAL has been shown to be better when it is performed early after the onset of pulmonary symptoms. In a series of 297 HSCT patients who underwent a BAL, the diagnostic yield of the procedure was 56.8 % in patients since less than 24 h versus 32.8 % in the others [131]. In another study, the diagnostic yield was 73 % in patients who underwent BAL within 4 days of presentation and 31 % thereafter [2]. This may be due to the effect of previous anti-infectives on the probability to identify a pathogen, but also to the fact that lung inflammatory lesions may persist some time after the

infection is controlled, so that delayed BAL may be performed in patients with a favorable outcome but still imaging and clinical signs. Therefore, it is recommended to do a BAL as soon as possible.
– Finally, although pneumonia is less frequent after autologous than after allogeneic HSCT, the diagnostic yield of BAL has been reported to be lower in pneumonia occurring after autologous rather than after allogeneic HSCT [133].

However, despite these variabilities, BAL, when well tolerated and correctly processed at the laboratory, represents the best diagnostic strategy for a minimum of complications. It should also be noticed that cytologic examination of BAL fluid will also document alveolar hemorrhage [96] or alveolar proteinosis [99].

A routine BAL protocol for HSCT recipients should include at least total and differential cell counts on cytocentrifuge preparations using May-Grünwald-Giemsa stains, as well as cytologic examination on cell pellets obtained by centrifugation and cytocentrifugation that are stained with the May-Grünwald-Giemsa stains and the Papanicolaou stain for viruses and the Gomori-Grocott method for *P. jirovecii* and fungi (Table 16-1). Other stains are necessary to identify alveolar proteinosis (PAS) [99], mycobacteria (Zielh), and siderophages (Perls' Prussian blue) [96].

A sample of fluid should be sent for bacteriologic and fungal cultures and viral tests. Galactomannan detection may be done in BAL fluid, especially in neutropenic patients with aspergillosis [128, 134], but with a higher cutoff (≥1) than in serum [121]. Aspiration and BAL fluids should be examined for *Legionella pneumophila* by cultures and eventually NAT and for *Nocardia* and mycobacteria. Due to the better sensitivity of qPCR over conventional stainings and IF assays [54, 55, 59], some laboratories already use qPCR exclusively. The viruses of interest in HSCT patients are the viruses of the herpes family, adenoviruses, and respiratory viruses (i.e., respiratory syncytial virus, influenza, and parainfluenza, rhinoviruses, metapneumoviruses, coronaviruses, enteroviruses, and bocavirus) which should be determined particularly in the setting of known exposures and during seasonal outbreaks [79].

A *protected bacteriologic sample* (*PBS*), done by a protected brush specimen or a plugged telescoping catheter, should be processed by quantitative culture techniques. Although determined from mechanically ventilated patients, the minimal threshold bacterial concentration required to usually consider the isolated pathogen as the cause of the pneumonia is 10^3 colony-forming units (CFUs)/mL for PBS and 10^4 to 10^5 CFUs/mL in the BAL fluid [135, 136].

Due to the increased risk it provides for bleeding and pneumothorax, *transbronchial biopsy* is not routine in acute pneumonia occurring in patients with HSCT and should not be proposed with the first bronchoscopy and BAL [137, 138]. Also, it does not add significant informations to concomitant BAL in most cases [133, 138, 139].

In cases in which noncontributory bronchoscopy, one should consider performing a second BAL and/or a transbronchial biopsy or better, a transthoracic needle aspiration when the lesion(s) is nodular and subpleural [126]. After HSCT, focal lesions that develop or persist despite antibiotics are mostly of fungal origin [140]. Successful fine needle aspiration, guided by either ultrasound or CT, has been reported, with a complication rate around 15 %, and is useful for documenting IFD when other procedures failed [140, 141]. The final decision between lung biopsy through open or video-assisted thoracoscopy or empirical treatment to cover the most likely organisms should be made by the transplant physician and the lung specialist after weighing the risks of surgery, empirical treatment, and failure to reach a diagnosis and the etiologies most likely at that time after transplantation. Lung biopsy is more helpful when the clinical course is prolonged and the pattern is nodular or cavitary.

16.5.3 Starting Treatment and Reevaluation of Efficacy

Because any pneumonia that occurs after HSCT may be life threatening, empirical antibiotics against the likely organisms must be started immediately. The best approach is to conduct bronchoscopic investigation with BAL as soon as possible; this should not, however, delay the initiation of treatment, especially when acute (likely bacterial) pneumonia is present or with patients who are neutropenic. Consideration should be given to the likelihood of fungus in patients with prolonged neutropenia and in those with GVHD on steroid therapy. Some empirical treatments may render subsequent testing negative, especially that for bacteria and viruses, yet they may be warranted. Some empirical treatments will not affect the chance of isolating the pathogen for at least several days after the empirical treatment is begun (e.g., TMP-SMX for *P. jirovecii*, antifungal agents for aspergillosis).

Daily clinical reevaluation should be performed, especially when no diagnosis is initially established and the patient does not improve. The use of noninvasive markers, when initially positive, is mostly useful to assess the treatment efficacy:

– Patients with initial positive blood cultures should be sampled for blood culture controls daily until negative.
– It has been shown in aspergillus infection with an initial positive serum or plasma galactomannan test that the quantitative evolution of the test correlates with the prognosis as soon as from the first week of therapy [142, 143].

Serial follow-up X-rays or, preferably, lung CT scans should be repeated according to the type and severity of the pneumonia. However, some infections, although favorably evolving, may be associated with a long persistence of image abnormalities, which may take several months to decrease or disappear. In the absence of new lesions, it should not be per

se a reason to reinvestigate the patient if the clinical outcome is favorable. In aspergillosis, it has been shown that a transient increase of the volume of the fungal lesions on CT scan may occur at the time of neutropenia recovery without any significance of treatment failure [112].

New investigations should be rapidly undertaken when the pneumonia does not respond to empirical treatment. Even when the cause of the pneumonia has been established, the occurrence of new infiltrates should be regarded as suspicious for treatment failure or new infections, as the association or succession of several causes of pneumonia is not uncommon in this setting. When a BAL has been initially done on accessible lesions, a second one should not be considered before most of the results of the laboratory be back, except if the BAL has been performed in poor conditions or in case of new lesions. Usually, a delay of 1 week before a first noncontributory BAL and a second BAL is minimal. If the initial lesion is peripheral and nodular and the BAL was noncontributive, a transthoracic fine needle biopsy should be considered. If the lesion is subacute or chronic and there is no response to targeted or empirical treatment, surgical biopsy may be contemplated for chronic nodular lesions.

16.6 Place of Intensive Care and Ventilatory Support

Pneumonia is the cause of the ICU transfer in roughly one third of the cases both in allogeneic [144] and autologous [145] HSCT recipients. Although the prognosis of HSCT patients transferred in the ICU has slightly increased over time [146], the decision of transfer remains difficult in terms of the emotional burden for the patient, family, and caregivers. The use of predictive scores—such as the sepsis-related organ failure assessment (SOFA) [147]—assessed at ICU transfer in HSCT recipients is debated [148]. Patients with acute respiratory failure benefit from ICU support and can be investigated by BAL, knowing that BAL does not increase the need for mechanical ventilation [149]. The prognosis of ICU support is usually better in autologous rather than in allogeneic HSCT recipients, and those with severe acute GVHD and under corticosteroids usually do not clearly benefit from ICU support [146]. Guidelines should be adapted to new data, but, in general, the clinician should consider the individual's chance of survival and of return to an acceptable life before transferring the patient to an ICU. The patient and the family should be provided with reasonable estimations of prognosis before transfer; in addition, the likelihood of continuing life support should be considered regularly during the course of treatment. Patients who respond to noninvasive mechanical ventilation have a better prognosis than those who required mechanical ventilation [150].

16.7 Summary

Pneumonia is a principal determinant of posttransplantation survival. Because of the predictable timing of some infections after most types of transplantations, some prophylactic regimens have been instituted with far-reaching benefits. However, any change in the transplant procedure, conditioning, or immunosuppressive regimen may affect the incidence and cause of infectious pneumonia. Additionally, new pathogens are emerging, and familiar pathogens are becoming more resistant. A high level of suspicion when pneumonia occurs in a transplant recipient and vigilance in diagnosing and treating will continue to be required to prevent an increase in mortality from pneumonia. The development of indirect diagnostic procedures is essential in the evaluation of pneumonia, but their clinical pertinence must be established in large prospective studies, and, until now, they do not replace direct investigation of the lung, mainly by BAL.

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Pneumonia After Solid Organ Transplantation

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Pneumonia in solid organ transplant (SOT) recipients encompasses a broad spectrum of disease caused by a diverse group of pathogens and may result in significant morbidity and mortality. The risk of pneumonia following SOT is influenced by many factors including the organ transplanted, the time from surgery, the net state of immunosuppression, and the presence of comorbid medical conditions. Pneumonia is particularly common following lung transplantation.

In this chapter we review the pathogenesis, risk factors, and epidemiology of pneumonia in SOT recipients with a focus on the major causative organisms and the specific risks associated with each SOT type. We also discuss the approach to the diagnosis and empiric treatment of SOT recipients with suspected pneumonia, examine strategies for preventing post-transplant lung infections, and highlight some of the future directions in the management of pneumonia in SOT recipients.

17.1 Pathogenesis

Host defenses against pulmonary infection include components of the innate immune system such as the airway epithelium and alveolar macrophages, as well as aspects of adaptive immunity such as T-cell and B-cell-associated responses to pulmonary pathogens. In SOT recipients, these defenses are affected by numerous pre-transplant, peri-transplant, and post-transplant risk factors for infection.

Prior to transplantation, SOT recipients may be chronically ill with multiple comorbid medical conditions, poor nutritional status, and the need for frequent hospitalization, which may all predispose to pulmonary infection. Pre-transplant pulmonary infections are particularly prevalent in patients being considered for lung transplantation, and colonization with certain bacteria may increase the risk of post-transplant pneumonia. For example, among patients with cystic fibrosis, prior pulmonary infection or colonization with *Pseudomonas* may be predictive of post-transplant infection [1], and pre-transplant colonization with certain species of *Burkholderia cepacia* complex may be associated with higher rates of postoperative infection and death [2–4]. In general, however, Gram stain of donor respiratory specimens correlates poorly with the development of post-transplant pneumonia in lung transplant recipients [5].

An important peri-transplant risk factor for pulmonary infection is the need for endotracheal intubation [6], which may be prolonged following complex surgeries. For example, one review of 546 liver transplant recipients in Germany reported that 11 % of patients required endotracheal intubation for more than 24 h after surgery [7]. Postoperative intubation may also be prolonged following lung and heart transplantation although strategies for early extubation in these patients are being investigated [8–10].

In the post-transplant setting, the risk of pneumonia is strongly influenced by the introduction of transplant immunosuppression. Although most lung infections in SOT recipients are due to common bacteria [11, 12], the impaired cell-mediated immunity resulting from combination immunosuppression also results in increased rates of infection with viruses, fungi, intracellular bacteria, and mycobacteria.

The calcineurin inhibitors (cyclosporine and tacrolimus) cause reduced levels of TNF-alpha, IL-2, and other inflammatory cytokines, resulting in impaired lymphocyte proliferation and defects in T-cell and B-cell immune responses. These drugs have been linked with increased rates of fungal pneumonia and infections due to cytomegalovirus (CMV), *Pneumocystis* and *Legionella*. The mTOR inhibitors (sirolimus and everolimus) interrupt T and B cell responses to inflammatory cytokines, similarly resulting in impaired lymphocyte proliferation. Sirolimus has been associated with higher rates of pulmonary infection when compared to the calcineurin inhibitors [13] although the rate of CMV infection is reportedly lower in those receiving sirolimus. Antimetabolite therapies such as azathioprine and mycophenolate mofetil (MMF) impair lymphocyte proliferation and immunoglobulin production by blocking

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purine synthesis. MMF has been associated with lymphopenia and high rates of viral infection although it may be protective against the development of *Pneumocystis* pneumonia (PCP) in SOT recipients. Corticosteroids, the other mainstay of transplant immunosuppression, are broadly immunosuppressive and may result in increased rates of bacterial, viral, and fungal pneumonia including PCP.

There are also several unique considerations in the pathogenesis of pneumonia in lung transplant recipients. Pulmonary infection in lung transplant recipients may be transmitted directly from the donor [14]. Allograft rejection resulting in bronchiolitis obliterans syndrome (BOS) may further predispose to infection [15]. Additionally, lung transplant recipients may have an impaired cough reflex and be unable to clear pathogens from the airway [16], and the lack of intact lymphatic drainage may further impair the immune response.

17.2 Epidemiology

The rates of pulmonary infection and the causative pathogens vary greatly depending on the organ transplanted, the time from transplant, and the use of prophylactic antimicrobials. Pneumonia is the most common infectious complication following heart and lung transplantation [17–20], and the second most common infection after liver transplantation [21], but it occurs less frequently among kidney transplant recipients [22].

17.2.1 Time from Transplant and the Role of Prophylaxis

The typical timing of the onset of infection in SOT recipients has been well described [23, 24]. The post-transplant timeline is classically divided into three periods of varying infectious risk: the first month, month two to six, and more than six months after SOT. This timeline, however, is not absolute, and rates of infection are affected by other factors including the use of antimicrobial prophylaxis and the need for increased immunosuppression in cases of graft rejection.

In the first month following SOT, patients are most at risk for pulmonary infection due to hospital acquired pathogens. Healthcare-associated bacterial pneumonia may occur in recipients of any SOT during this period; however, bacterial pneumonia is most common in lung transplant recipients in the first month following transplant [18, 25]. Donor-derived bacterial and fungal pneumonia in lung transplant recipients may also present during this time.

In months two through six after SOT, pulmonary infection due to respiratory viruses, herpes viruses, and PCP are more common although the risk of CMV pneumonitis and PCP is reduced with prophylaxis. Pulmonary tuberculosis, typically due to reactivation and less commonly due to donor-derived infection in lung transplant recipients, may also present during this period. The median time to development of active tuberculosis following SOT is about 6 months [26].

Beyond six months after SOT, the risk of pneumonia varies depending on the ongoing need for immunosuppression. In patients for whom immunosuppression can be reduced, the risk of opportunistic infection typically declines, but patients remain at risk for community-acquired bacterial or viral pneumonia. In patients that still require high doses of immunosuppression due to rejection, the risk for opportunistic infections persists. Fungal pneumonia and pulmonary tuberculosis may occur during this time, and late-onset CMV pneumonitis or PCP may develop during this period after the discontinuation of prophylaxis.

17.2.2 Lung Transplant

Pneumonia occurs frequently following lung transplantation: one large Spanish study reported an annual incidence of pneumonia of 72 % among lung transplant recipients. In the immediate post-transplant period, particularly during the first postoperative month, bacterial pneumonia predominates [18, 25]. Gram-negative rods are the most common causative agents including *Pseudomonas*, *Acinetobacter*, *Stenotrophomonas*, and the Enterobacteriaceae although Gram-positive organisms such as *Staphylococcus aureus* also cause pneumonia in this population [25]. The majority of early bacterial pneumonia in lung transplant recipients is thought to be due to either hospital-acquired or donor-derived infections although the risk of donor-derived infection can be reduced with the use of appropriate targeted perioperative antibacterial prophylaxis [14]. Postoperative bacterial pneumonia, especially due to *Pseudomonas*, is particularly common among patients who undergo lung transplantation for cystic fibrosis. The increased rate of bacterial pneumonia in these patients may be the result of pre-transplant colonization with resistant organisms [27] as well as ongoing chloride channel defects and mucus production in the native upper airways.

About six months after transplant, lung transplant recipients may develop pneumonia due to community-acquired bacteria including *Streptococcus pneumoniae*, *Haemophilus influenza*, and *Legionella*. Those who develop chronic allograft rejection and BOS remain at risk for infection with *Pseudomonas* although it is unclear if *Pseudomonas* infection in these patients is a cause or result of BOS [28, 29].

Lung transplant recipients are also at risk for viral pneumonia during the post-transplant period, and viral infections have also been associated with rejection and BOS. CMV is a frequent and potentially severe cause of pulmonary infections in this population. Before the era of routine CMV prophylaxis, the incidence of CMV infection in high-risk lung transplant recipients was reported as high as >90 %, and high rates of CMV pneumonitis and death due to CMV were also described [30, 31]. CMV infection and pneumonitis have

both been associated with the development of BOS [32, 33] and higher post-transplant-mortality [34]. The rates of CMV infection and pneumonitis have been reduced by the widespread use of valganciclovir prophylaxis in high-risk patients [30, 35], and it is postulated that CMV prophylaxis may also help reduce the incidence of BOS in these patients [36].

In addition to CMV, lung transplant recipients are at risk for pulmonary infection with other herpes viruses and with community-acquired respiratory viruses. Herpes simplex virus may cause severe pneumonitis in this population but has become infrequent in the era of routine antiviral prophylaxis. Epstein–Barr virus (EBV) infection may result in post-transplant lymphoproliferative disease (PTLD), which may involve the lung and mimic pneumonia. Human herpes virus 6 (HHV-6) has been described as a cause of pneumonitis in SOT recipients and has also been associated with the development of BOS in lung transplant recipients [37] although this association has not been uniformly demonstrated [38]. Community-acquired respiratory viruses including influenza, respiratory syncytial virus (RSV) and adenovirus account for more than half of the respiratory infections in symptomatic outpatient lung transplant recipients [39, 40] and have also been inconsistently linked to rejection [41, 42].

Invasive fungal infections, especially invasive aspergillosis, were historically common in lung transplant recipients but have become less frequent with the use of antifungal prophylaxis [43]. A 2003 review reported a 6.2 % median incidence of *Aspergillus* infections in lung transplant recipients, which occurred a median of 3.2 months after transplant and were associated with 52 % overall mortality [44]. *Aspergillus* may cause invasive pulmonary infection in lung transplant recipients, or infection may be limited to tracheobronchitis. Airway colonization with *Aspergillus* has been liked to chronic rejection [45]. Pulmonary infection with other fungi including the agents of mucormycosis *Cryptococcus*, *Fusarium*, and the endemic mycoses are less common but have also been reported in lung transplant recipients.

Prior to the routine use of prophylaxis, PCP was a common cause of pneumonia in lung transplant recipients. One series reported an incidence of 88 % in heart–lung recipients [46], while another review described a 33 % incidence following lung transplantation [47]. Prevention of PCP following SOT with trimethoprim-sulfamethoxazole (TMP-SMX) is effective and typically recommended lifelong after lung transplantation as late PCP infections may occur following discontinuation of prophylaxis [47, 48].

Pulmonary tuberculosis occurs rarely in lung transplant recipients and may be the result of donor-derived infection, reactivation of latent infection, or primary infection post-transplant. Lung transplant recipients have been found to have higher rates of post-transplant tuberculosis than other organ recipients although this incidence varies depending on geographic location and other donor and recipient risk factors. Other rare causes of pneumonia following lung transplant include non-tuberculous mycobacteria, *Nocardia*, and parasites such as *Strongyloides* and *Toxoplasma*.

17.2.3 Heart Transplant

The incidence of pneumonia following heart transplant has been reported as 17–21 % [20, 49]. Pneumonia is the most common infection and most common respiratory complication following heart transplantation [20, 50]. About half of all respiratory infections in heart transplant recipients are caused by bacteria [20, 50]. Similar to lung transplant recipients, heart transplant recipients are at risk for healthcare-associated bacterial pneumonia in the immediate post-transplant period. Community-acquired bacteria and respiratory viruses become more common in the following months.

CMV frequently causes disease, including pulmonary infection, in heart transplant recipients not receiving prophylaxis. In a 1998 Spanish study of pneumonia following heart transplantation, CMV was the second most common pathogen isolated following bacteria [49]. High rates of death have been reported in heart transplant recipients with CMV pneumonitis [51]; however, CMV prophylaxis with valganciclovir has been shown to be safe and effective in this population [52].

Fungal pneumonia is also common following heart transplantation. Invasive pulmonary aspergillosis has been reported in 3.3–14 % of heart transplant recipients and accounts for the majority of invasive fungal infections following heart transplantation [53].

Prior to the use of prophylaxis, PCP was reported in up to 41 % of heart transplant recipients [54], but PCP has been shown to be effectively prevented by TMP-SMX prophylaxis in this population [54].

Toxoplasmosis is of particular concern in heart transplant recipients given that up to 75 % of seronegative heart transplant recipients from seropositive donors may develop disease in the absence of prophylaxis [55]. Pulmonary manifestations including fever, cough, and diffuse infiltrates on chest imaging may be the only manifestation of *Toxoplasma* infection in transplant recipients [55]. Donor-derived *Toxoplasma* infection can be prevented by administering prophylaxis to high-risk patients.

17.2.4 Liver Transplant

Postoperative pneumonia has been reported in up to 23 % of liver transplant recipients [56, 57]. Similar to other organ recipients, the majority of pulmonary infections in this population are due to bacteria [58]. High rates of Gram-negative and *Legionella* pneumonia have been reported in liver transplant recipients [58, 59] although these may now be less common in the setting of TMP-SMX prophylaxis.

The risk of respiratory complications following liver transplantation is greater among patients with more advanced liver disease [60], possibly due to volume overload, encephalopathy resulting in aspiration, and hepatopulmonary syndrome [19]. In the immediate post-transplant period, diaphragm dysfunction, poor nutritional status, and the need for prolonged intubation may also cause higher rates of pneumonia [19].

Similar to other SOT recipients, liver transplant recipients may also develop pulmonary infections with opportunistic pathogens including *Aspergillus*, CMV, and *Pneumocystis* [61] although some of these infections are preventable with prophylaxis.

Cryptococcus neoformans is a notable cause of pulmonary infection both before and after liver transplantation. Cirrhosis has been identified as a major risk factor for cryptococcal infection. In some series, 6–21 % of patients with cryptococcosis had underlying cirrhosis [62]. Although pre- and post-liver transplant recipients may develop disseminated cryptococcosis [63, 64], disease is limited to the lungs in about one-third of SOT recipients with cryptococcal infection [64]. Among SOT recipients with cryptococcal infection, isolated pulmonary infection may be more common in those receiving calcineurin inhibitors [64]. Calcineurin inhibitor use in SOT recipients with cryptococcosis has also been associated with lower overall mortality [64].

17.2.5 Kidney Transplant

The incidence of post-transplant pneumonia following kidney transplantation is lower than that following lung, heart, or liver transplantation, possibly due to the less invasive nature of the surgery and lower acuity and severity of illness in kidney transplant recipients [65]. One large review found that hospitalization for pneumonia occurred 2.86 times per 100 person-years in kidney transplant recipients [66], while another found that 13 % of patients developed pneumonia in the first year following kidney transplantation [67]. Although postoperative pneumonia is less common among kidney transplant recipients, the specific pathogens involved and timing of infection are similar to other SOT recipients.

17.3 Noninfectious Conditions That May Mimic Pneumonia

Several noninfectious conditions with clinical or radiographic features similar to pneumonia may occur in SOT recipients. Diffuse pulmonary infiltrates may be due to pulmonary edema, transfusion-related lung injury, or acute respiratory distress syndrome. A focal pulmonary lesion may be caused by a pulmonary embolism, infarct, or malignancy.

In lung transplant recipients, graft rejection and BOS may result in respiratory symptoms and diffuse pulmonary infiltrates on chest imaging. Although BOS is often associated with infection, rejection may develop without concurrent infection.

Another noninfectious pulmonary complication that may occur in SOT recipients is the development of interstitial pneumonitis following treatment with the mTOR inhibitors sirolimus and everolimus. Patients with pneumonitis due to mTOR inhibitors may present with signs of pulmonary infection including cough, fever, and abnormal chest imaging. In one review of 217 patients who received sirolimus, 11 % developed pneumonitis [68], and in another review of 102 everolimus recipients, 12.7 % developed pulmonary toxicity [69]. Impaired renal function and a late switch to sirolimus as opposed to de novo use following transplant have been identified as risk factors for pulmonary toxicity in kidney transplant recipients [68, 70]. Symptoms typically resolve after mTOR inhibitor discontinuation [68, 69].

The incidence of lung cancer is higher among SOT recipients than the general population [71], particularly among former smokers and lung transplant recipients who undergo transplantation due to COPD [72]. Additionally, post-transplant lymphoproliferative disorder with pulmonary involvement may occur following any type of SOT transplantation and may be difficult to distinguish from an indolent infection without a tissue diagnosis.

17.4 Diagnosis

17.4.1 History and Physical Exam

The clinical manifestations of pneumonia in SOT recipients are variable due in part to the effects of immunosuppression and the wide range of pathogens that cause respiratory infections in this population. Some patients with pneumonia may present with fever, cough, and sputum production; however, typical symptoms may be absent in SOT recipients, particularly those infected with opportunistic pathogens.

The duration and severity of symptoms may be important clinical clues. Patients with bacterial pneumonia typically present with an acute onset illness, while fungal, mycobacterial or PCP may present with a more indolent or chronic onset illness. Chronic cough, persistent fever, or unexplained hypoxia may be the only clinical signs of infection in some patients. Hemoptysis may suggest an invasive fungal or mycobacterial infection. Extrapulmonary symptoms such as nausea, vomiting, diarrhea, rash, sore throat, or myalgia may suggest a viral etiology such as influenza or CMV.

The evaluation of SOT recipients with suspected pneumonia should also include a careful assessment of sick contacts, travel history, animal exposure, and tobacco and drug use.

Typical physical exam signs of pulmonary infection such as rales may also be lacking in SOT recipients. For example, pulmonary auscultation may be normal in patients with PCP. The initial examination should also include evaluation for signs of systemic infection including conjunctivitis, skin findings, lymphadenopathy, hepatosplenomegaly, or abdominal tenderness.

17.4.2 Imaging

Chest X-ray is the initial imaging test for most SOT patient with suspected pneumonia. Bacterial pneumonia including *Legionella* may manifest as a focal consolidation, while viral infections and PCP are typically more diffuse or not visualized

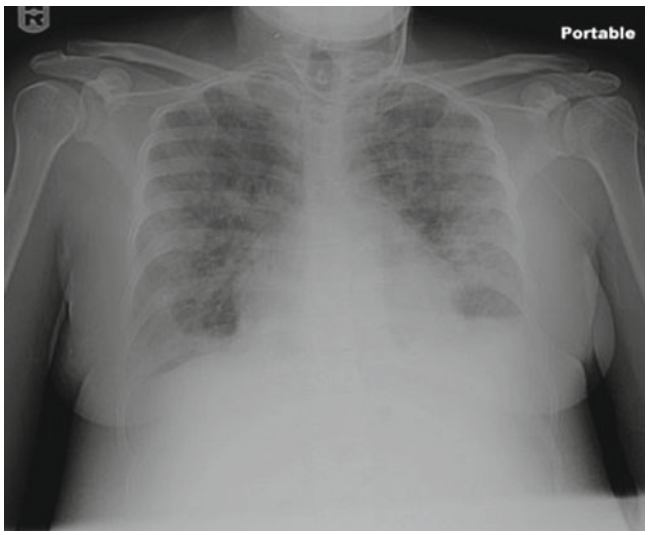

FIGURE 17-1. Chest X-ray of PCP in a liver transplant recipient.

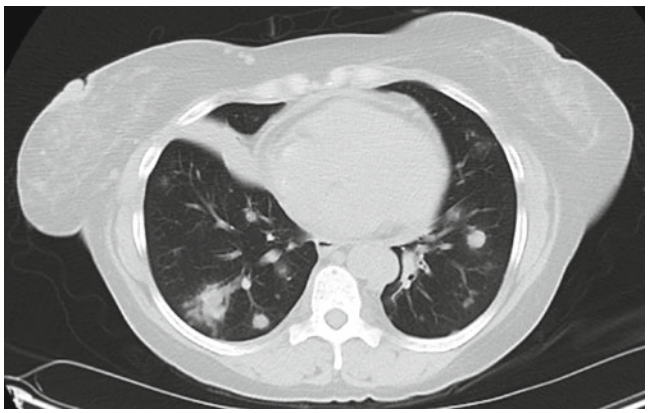

FIGURE 17-2. Chest CT of a kidney transplant recipient with invasive pulmonary aspergillosis.

at all (Figure 17-1). Although tuberculosis is classically described as producing bilateral apical infiltrates, chest X-ray findings may be variable among SOT recipients. The appearance of fungal pneumonia on X-ray imaging is also variable and includes nodular lesions, cavitary disease, or patchy infiltrates. Because invasive fungal infections may be poorly visualized with X-ray imaging, computed tomography (CT) imaging is the preferred modality to evaluate for fungal pneumonia in these patients.

CT imaging of the chest may provide important diagnostic information in the evaluation for pneumonia in SOT recipients. Bacterial pneumonia is usually readily visible on CT imaging. Invasive pulmonary aspergillosis in SOT recipients usually causes nodular or mass-like pulmonary lesions [44, 73] (Figure 17-2). The "halo sign" and the "air-crescent sign," which are classically described CT findings of invasive pulmonary aspergillosis in patients with hematologic malignancies, may be absent in the SOT population [44, 73]. Pulmonary mucormycosis may also be seen as a nodular or cavitary lesion on CT [74]. CT imaging may also reveal the diffuse infiltrates of a viral pneumonia or PCP (Figures 17-3 and 17-4). Additionally, CT may identify noninfectious causes of pulmonary disease such as malignancy or pulmonary emboli and may provide important information regarding the anatomic location of the suspected infection to help determine if the area is amenable to bronchoscopic evaluation.

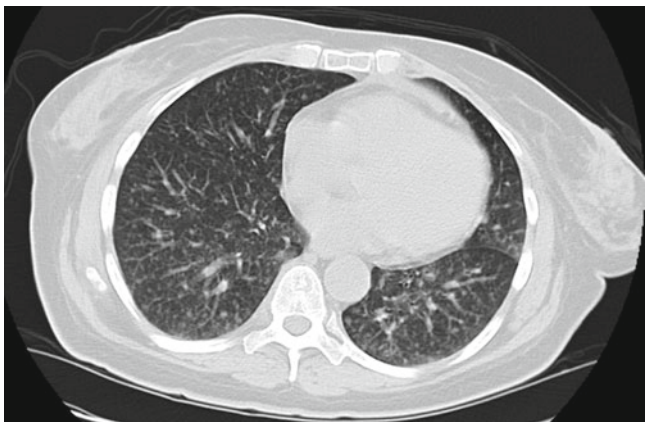

FIGURE 17-3. Chest CT of a liver transplant recipient with CMV pneumonitis.

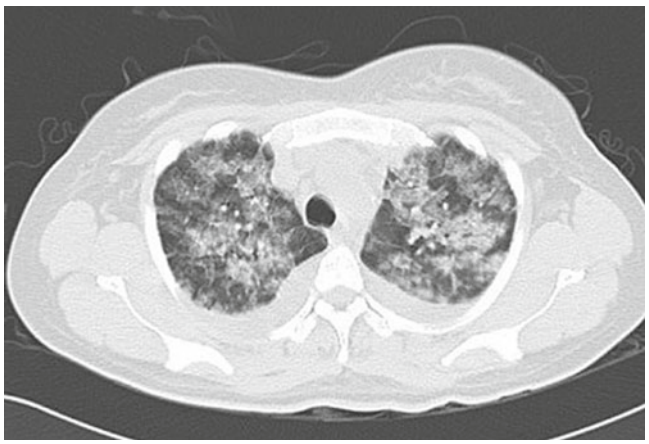

FIGURE 17-4. Chest CT of PCP in a liver transplant recipient.

17.4.3 Blood and Urine Testing

Blood and urine testing may provide valuable diagnostic information for SOT recipients with suspected pneumonia, and in some cases these tests may definitively establish a causative organism.

Blood cultures are positive in about 4–18 % of patients with community-acquired bacterial pneumonia [75]. For those with negative blood and sputum cultures, urine antigen testing for *Streptococcus pneumoniae* and *Legionella* may helpful. These tests have been shown to be insensitive, but highly specific [76, 77] although the *Legionella* urinary

antigen test is only able to diagnose infection with *Legionella pneumophila* serogroup 1. Urinary antigen excretion may be prolonged in immunocompromised patients with Legionnaire's disease [78].

In patients with suspected viral pneumonia, serum antigen or polymerase chain reaction (PCR) testing may provide diagnostic insight. Serum CMV DNA PCR testing is often performed in patients with suspected CMV pneumonia. Although serum CMV DNA viral loads do not strongly correlate with the existence of CMV pneumonia [79], this test may suggest the presence of invasive infection when levels are significantly elevated. Other viral causes of pneumonia in SOT recipients such as adenovirus and HSV may also be identified via serum PCR testing.

Serum testing is an important adjunct in the diagnosis of invasive fungal pneumonia. Serum galactomannan is specific but not sensitive for the detection of invasive aspergillosis in SOT recipients. One large meta-analysis reported that serum galactomannan was 89 % specific and 71 % sensitive for diagnosing proven aspergillosis, but the same study found a sensitivity of only 22 % in SOT recipients. False-positive galactomannan results may occur in patients receiving piperacillin-tazobactam or amoxicillin-clavulanic acid, those who consume foods contaminated with mold, and those with infections due to other fungi that may cross-react with the test. False-positive results are particularly common in the immediate post-transplant period and in lung transplant recipients [80, 81]. False-negative results may occur in patients receiving mold-active antifungal prophylaxis.

The 1,3-Beta-D-glucan assay is another serum test useful for the diagnosis of invasive fungal pneumonia. Unlike galactomannan, 1,3-Beta-D-glucan is not specific for aspergillosis, and elevated levels may also occur during infection with PCP, *Candida*, *Fusarium*, and *Histoplasma* but not *Cryptococcus* or mucormycosis. As a result, a positive 1,3-Beta-D-glucan does not establish a specific diagnosis but may provide useful information in the appropriate clinical context. In the diagnosis of PCP, 1,3-Beta-D-glucan has been found to be highly sensitive although data in SOT recipients is limited [82]. False-positive results may occur in patients on hemodialysis, those receiving intravenous immunoglobulin or albumin, and those with *Pseudomonas* bacteremia.

Antibody testing or serum and urinary antigen testing for endemic mycoses may also be useful in patients with risk factors and signs of pulmonary infection with these organisms. For example, in one large review of 152 cases of histoplasmosis in SOT recipients, 80 % of whom had pulmonary disease, serum antigen testing was positive in 86 %, and the urine antigen was positive in 93 % [83]. Another study of coccidioidomycosis in SOT recipients found that individual serologic tests were positive in 21–56 % of cases, but when multiple tests were used for the same patient, at least one was positive in 77 % of cases [84].

17.4.4 Expectorated Sputum and Nasopharyngeal Testing

Expectorated sputum is of limited value in the diagnosis of pneumonia following SOT particularly among lung transplant recipients, whose airways are frequently colonized with *Pseudomonas* and other bacteria. However, expectorated sputum stains and cultures may be useful when cultures reveal an organism compatible with the clinical syndrome such as *Streptococcus pneumoniae* or an organism that should typically be considered pathogenic such as *Mycobacterium tuberculosis* or *Pneumocystis*.

Nasopharyngeal swab collection for viral detection via culture, DFA or PCR may be useful for identifying upper respiratory viral pathogens although positive nasopharyngeal viral cultures could represent colonization as opposed to true infection, and these tests may fail to identify lower respiratory tract disease, which is better evaluated via bronchoscopy [85].

17.4.5 Bronchoscopy

Bronchoscopy with bronchoalveolar lavage (BAL) and in some cases transbronchial biopsy is a mainstay in the diagnostic evaluation of SOT recipients with pneumonia. Several studies have shown that bronchoscopy can be a safe and effective method for identifying the cause of pulmonary infection in the SOT population [86, 87]. Furthermore, in lung transplant recipients with suspected pneumonia, biopsy is essential to evaluate for BOS and rejection. Biopsy may also be necessary to demonstrate the presence of an invasive fungal infection and to diagnose a pulmonary malignancy.

BAL fluid can be analyzed for Gram stain and bacterial culture, mycobacterial studies, viral PCR and culture, *Pneumocystis* PCR and stains, fungal stains and culture, and stains for parasitic infection. Multiplex PCR testing has been shown to enhance the diagnosis of viral pneumonia in lung transplant recipients [88]. Galactomannan testing may be performed on BAL fluid and when used in conjunction with fungal cultures may improve the sensitivity and specificity of detecting invasive aspergillosis in SOT recipients [89]

Cytology may also be performed on cells collected during BAL for evidence of malignancy, PCP, or other fungal infections.

A frequent diagnostic dilemma following bronchoscopy is differentiating between airway colonization and active infection when an organism is identified. The airways of lung and other SOT recipients often become colonized with drug-resistant bacteria and fungi; however, the isolation of these organisms may not indicate infection. *Candida*, in particular, is frequently isolated from respiratory specimens but is rarely thought to be a cause of invasive disease with the exception of lung transplant patients with suspected anastomotic *Candida* tracheobronchitis [90]. Similarly, viral cul-

tures from BAL fluid may be positive in the absence of histologic evidence of tissue invasion. In these situations, the BAL findings must be interpreted in the context of other available clinical data.

17.5 Empiric Treatment

The most important aspect of treating pulmonary infections in SOT recipients is to establish the diagnosis. Determining the causative organism is ideal, but not always possible or timely, and therefore empiric therapy is usually initiated early in the diagnostic workup. The approach to treating specific pathogens is beyond the scope of this chapter and is detailed elsewhere in this book.

Empiric antibiotic choices for bacterial pneumonia in SOT should be based on national guidelines for community-acquired and healthcare-associated pneumonia [91, 92] but should also be guided by patient-specific respiratory culture data when possible. Choosing appropriate empiric therapy may be particularly challenging in lung transplant recipients who are more frequently colonized with multidrug-resistant Gram-negative organisms.

Empiric antiviral therapy may be initiated with oseltamivir for suspected influenza and with either ganciclovir or foscarnet for suspected CMV pneumonitis. Intravenous TMP-SMX (with corticosteroids when severe hypoxia is present) may be initiated while awaiting confirmation of PCP.

SOT recipients with presumed invasive fungal pneumonia may be treated with either a lipid amphotericin B formulation or voriconazole when there is a high suspicion for pulmonary aspergillosis. Of note, voriconazole is not effective for the treatment of mucormycosis and must be used with caution in SOT recipients due to the risk of serious drug interactions. Voriconazole is a potent inhibitor of several of the cytochrome P450 enzymes, which may result in decreased metabolism of the calcineurin inhibitors tacrolimus and cyclosporine. Calcineurin inhibitor doses need to be adjusted and levels monitored closely when co-administering voriconazole.

Empiric therapy for pulmonary infection in SOT recipients should be adjusted based on culture and imaging data, and the patients' clinical response to therapy. A lack of response to empiric treatment should prompt a re-evaluation of the treatment strategy and further investigation into the etiology of the infection.

17.6 Prevention

The epidemiology of pneumonia in SOT recipients is changing due to the evolution of preventive strategies, which primarily consist of vaccination and antimicrobial prophylaxis.

Vaccination for measles, mumps, rubella, diphtheria, and pertussis is recommended for all patients prior to SOT [93]. Live vaccines including the measles, mumps and rubella vaccine, varicella vaccine, and live attenuated influenza vaccine, are contraindicated following SOT, and should generally be given at least four weeks prior to transplantation.

Yearly influenza vaccination with the inactivated vaccine is recommended following SOT [93]. Although the reported immunogenicity of the inactivated influenza vaccine in SOT recipients has been variable, influenza vaccination in this population has generally been found to be safe and effective [94, 95]. In one large review of 51,730 kidney transplant recipients of whom 9678 of whom received influenza vaccination, receipt of vaccine was associated with lower rates of graft loss and death [96]. Early reports of a possible association between influenza vaccination and rejection have not been supported by larger studies [94, 95]. Yearly influenza vaccination of household members of SOT recipients is also recommended [93]

Vaccination against *Streptococcus pneumoniae* is also recommended prior to SOT transplantation. The current recommendation is to administer the 13-valent protein-conjugated (PCV13) vaccine first, followed by the 23-valent polysaccharide vaccine (PPSV23) at least eight weeks later with an additional dose of PPSV23 given five years later and after age 65 [97]. Some experts recommend monitoring yearly pneumococcal antibody titers in SOT recipients given evidence of waning immunity in this population and the potential need for further PPSV23 doses [93, 98]. The PCV13 and PPSV23 vaccines have both been found to prevent invasive and noninvasive pneumococcal disease in the general population, but studies of pneumococcal vaccine efficacy in SOT recipients are limited. However, a study of the seven-valent protein-conjugated vaccine in HIV-infected adults showed that it was effective in this population [99], which suggests that protein-conjugated pneumococcal vaccination may be efficacious for other immunocompromised patients as well.

Antimicrobial prophylaxis strategies following SOT are typically designed to prevent PCP and CMV disease. PCP prophylaxis with TMP-SMX is usually given for 6–12 months following SOT, but should be considered for longer durations in all transplant recipients and particularly in patients with a history of PCP infection, small bowel or lung transplant recipients, and patients being treated for rejection [100]. TMP-SMX is highly effective for PCP prevention. In a Cochrane review of PCP prevention in non-HIV patients, TMP-SMX use was associated with an 85 % reduction in the incidence of PCP infection [101]. TMP-SMX is considered the drug of choice in SOT recipients not only because of its efficacy against PCP, but also because it may confer additional protection against *Toxoplasma*, *Legionella*, *Listeria*, *Nocardia*, and other bacteria. Alternative drugs for PCP prophylaxis such as atovaquone and dapsone are less protective against PCP and do not provide the same broad protection against other pathogens.

CMV prophylaxis is routinely administered to high-risk SOT recipients usually for 3–6 months and up to 12 months in lung transplant recipients. The major risk factor for CMV

disease is CMV IgG seronegative recipients and seropositive donor (D+/R−) status. CMV IgG seropositive recipients (D+/R+ and D−/R+) are also at risk for CMV disease. The incidence of post-transplant CMV in D−/R− SOT recipients is significantly lower, and prophylaxis is generally not indicated for these patients. Although valganciclovir prophylaxis is effective, CMV disease may still occur following the cessation of prophylaxis. For example, in a study of D+/R− kidney transplant recipients, CMV disease in the first two years following transplant occurred in 39 % of patients who received 100 days of valganciclovir prophylaxis and 21 % who received 200 days [102]. Due to high rates of late-onset CMV disease in lung transplant recipients, indefinite CMV prophylaxis for these patients has also been investigated, but high rates of valganciclovir discontinuation due to hematologic toxicity have been reported with indefinite use [103].

An alternative strategy for CMV disease prevention is preemptive therapy, which entails monitoring patients for CMV viremia with weekly blood tests following SOT and then initiating antiviral therapy in those with detectable viremia before symptomatic disease develops [104]. Although this approach has been shown to be as effective as routine prophylaxis for the prevention of CMV disease in patients at intermediate risk for CMV, the efficacy of preemptive therapy for the highest risk patients is still under investigation [104].

In addition to PCP and CMV prophylaxis, lung transplant recipients often receive postoperative antibacterial prophylaxis for 3–7 days. Many lung transplant centers also administer universal antifungal prophylaxis either with inhaled amphotericin or systemic voriconazole to prevent invasive aspergillosis [105].

Heart transplant recipients at risk for toxoplasmosis such as those who are seropositive for *Toxoplasma* and those who are seronegative and receive an organ from a seropositive donor should receive *Toxoplasma* prophylaxis [55]. TMP-SMX is also the recommended prophylactic medication for *Toxoplasma* although other regimens such as dapsone, atovaquone, or sulfadiazine, each with pyrimethamine, are also used at some centers [55].

17.7 Future Directions

Several aspects of the diagnosis, treatment and prevention of pneumonia in SOT recipients are currently under investigation. Innovations in diagnostics are aimed at more accurate, faster, and less invasive identification of bacterial, viral, and fungal infections. Novel antibiotics are being developed that may be effective for the treatment of multidrug-resistant Gram-negative pneumonia, particularly among cystic fibrosis patient who undergo lung transplantation. New azole antifungal agents are being investigated for the treatment of invasive aspergillosis and other fungal infections. Innovative preventive strategies, particularly for CMV and vaccine preventable illness, are also being evaluated in clinical trials.

17.8 Conclusion

Pulmonary infections in SOT recipients are caused by a wide range of pathogens and result in a broad spectrum of clinical disease. Some of the usual causes of pneumonia are effectively prevented with routine postoperative prophylaxis, but nonetheless, pneumonia is common in this population, particularly among lung transplant recipients. When pneumonia is suspected, a thorough investigation for the causative pathogen is essential. Diagnosis usually requires imaging, microbiologic testing, and often bronchoscopy. Although empiric therapy for pneumonia is often clinically appropriate, establishing the etiology of infection is essential to ensure appropriate treatment and effective cure. The investigation of novel diagnostic, therapeutic, and preventive strategies for pneumonia in SOT recipients is ongoing, and the management of these patients will continue to evolve as new data emerges.

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18
Central Nervous System (CNS) Infections After Hematopoietic Stem Cell or Solid Organ Transplantation

Diana Averbuch and Dan Engelhard

18.1 Central Nervous System (CNS) Complications After Transplantation

CNS complications are frequent after stem cell transplantation (HSCT) and solid organ transplantation (SOT). Among HSCT patients, 10–59 % develop CNS complications [1–5]. The type of the HSCT influences the incidence. For example, in one study, the incidence of CNS complications was highest in unrelated allogeneic transplantations (39 %), followed by related allogeneic (21 %) and autologous transplantations (11 %) [1]. Differential diagnosis includes infectious, metabolic, bleeding, drug induced, posterior reversible encephalopathy syndrome (PRES), and other etiologies [6, 7]. In one study in HSCT patients with reduced intensity conditioning (RIC), the etiologies of 31 episodes of neurological complications (23 CNS), were due to nonfocal encephalopathies ($n=11$), meningoencephalitis ($n=5$), and stroke or hemorrhage ($n=4$ each). The majority of them appeared before day +100. Drug-related toxicity was responsible for 10/31 events (32 %) (eight caused by cyclosporine) [5]. In another study, 10/77 (13 %) patients following umbilical cord transplantation developed early (the median time of onset 19 days (range: 2–58 days) CNS complications presented as impaired consciousness; etiologies included cyclosporine ($n=5$) or tacrolimus encephalopathy ($n=2$), thrombocytic microangiopathy ($n=1$), and unknown ($n=3$) [8]).

The frequency of neurological complications after SOT varies: 8–80 % in liver, 7–60 % in heart, 22–33 % in lung, and 21–30 % in renal transplant patients [9–13]. The possible etiologies include infections, drug toxicity, cerebrovascular events, bleeding, pontine myelinolysis, PRES, and others [12–14]. In one study, 64/395 (16.2 %) liver transplant patients developed major neurological symptoms (37 within 30 days of transplant), including cerebrovascular in 15 patients (3.8 %), tacrolimus-related leukoencephalopathy in 3 (0.8 %), pontine myelinolysis in 2 patients (0.5 %); no clear cause was identified for 44 cases (11.1 %) [12]. Neurologic complications were found in 75/384 (19.5 %) heart transplant patients; 32 % of infectious and 68 % of noninfectious etiology, including stroke in 25/78 (32 %); more rare complications were seizures, episodes of transient ischemic attack, anoxic encephalopathy, metabolic encephalopathy, and others [14]. Among patients after intestinal transplantation, 46/54 (68 %) developed headaches (50 %), encephalopathy (43 %), seizures (17 %), opportunistic CNS infections (7 %), and ischemic stroke (4 %) [15].

Neurologic complications were reported in 68 % of 132 lung transplant recipients, mainly impairment of consciousness (25 %), headaches (20 %), and neuromuscular complications (21 %). The neurologic complications were commonly related to immunosuppressant neurotoxicity (17 %) and opportunistic infections (11 %). There was a trend for an increased frequency of seizures and headaches in recipients with cystic fibrosis ($p>0.05$) [16].

CNS complications affect the outcome in transplantation. In one neuropathological autopsy study in HSCT, the survival time was almost half in the group (37/180 patients) of "CNS as cause of death" as compared to the rest 143 patients whose cause of death was considered to be extracerebral (96 vs. 178 days; $p=0.0162$). Infectious disorders were one of the two main neuropathological findings [17]. In HSCT RIC patients with CNS involvement, 1-year non-relapse mortality was significantly worse (42 % vs. 20 %, $p=0.02$) and 4-year overall survival less (33 % vs. 45 %, $p=0.05$) [5]. 4/10 umbilical cord transplant patients with early CNS complications died within 30 days [8]. Mortality rate in heart transplant patients was 12/53 (22.6 %) with noninfectious and 12 % with infectious neurological complications (42.8 % if the CNS was involved) [14]. On the other hand, the presence of neurologic complications did not affect posttransplant survival in one study in lung transplant patients [16].

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18.1.1 Incidence for CNS Infections

The incidence of CNS infections varies between 2 and 15 % in HSCT [4, 6, 17–22], 0.6 and 10 % in liver [23–25], 4 and 13 % in renal [11, 26], 1 and 3 % in lung [9, 11], and 3 and 18 % in cardiac transplant recipients [10, 27]. The outcome is usually grave: mortality up to 36–67 % is reported in HSCT [2, 6]. In heart transplant recipients, CNS infection was found to be a strong predictor for mortality in multivariate analysis (HR, 4.39; 95 % CI, 1.72–11.18; $p = 0.002$) [28].

18.1.2 Risk Factors for CNS Infections

CNS infections occur in allogeneic, and they are very rare in autologous HSCT [6, 19]. In the early post-HSCT period, there is severe neutropenia as an important risk factor if it is prolonged. The early post-engraftment period (30–100 days) is characterized by acute GVHD and cellular immune deficiency. Later on, chronic GVHD and its immunosuppressive therapy are major risk factors [21, 29, 30]. Following SOT, intensive immunosuppressive therapy early after transplantation, or in later periods, especially following rejection or retransplantation, is a major risk factor [31, 32].

18.1.3 Etiology of CNS Infections

Fungal pathogens, mainly *Aspergillus*, are the most common, up to 70 %, causes of CNS infections in HSCT and SOT [10, 17, 18, 26–28, 33–35]. Bacteria, on the other hand, are infrequent causes of CNS infections in SOT and HSCT, it causes 0–10 % of them [18, 28, 36]. The etiology depends on geographical prevalence of certain pathogens. For example, in one study from Germany, the most common CNS infections post-HSCT was *Toxoplasma* encephalitis (74 %), while only 18 % had *Aspergillus* and 4 % had each *Candida* and viral encephalitis [6]. Toxoplasmosis was also found in 30 % of 180 HSCT recipients, in an autopsy study in Brazil [18].

Effective antimicrobial prophylactic strategies have led to a change in the epidemiology of opportunistic CNS infections transplant recipients. A third of CNS infections post-heart transplantation in two cohorts from 1968 to 1987 were due to *Listeria monocytogenes* or *Toxoplasma gondii*, while these pathogens were not found in the later cohort in 2007 after the implementation of trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis [28, 33, 34]. There is a trend toward less viral infection, especially CMV, due to the application of PCR monitoring and preemptive treatment [6].

Table 18-1. Main etiologies of focal lesions vs. meningoencephalitis in CNS infectious diseases

Focal lesions	Meningitis/meningoencephalitis
Aspergillosis	Cryptococcosis
Zygomycosis	Herpes viruses
Dematiaceous fungi	Polyomaviruses (JC)
Nocardiosis	Listeriosis
Toxoplasmasmosis	
EBV (PTLD)	

18.1.4 Clinical Manifestations of CNS Infections

The clinical manifestations include fever, headache, altered mental status, focal neurological signs, seizures, and other signs. These signs may develop rapidly, as in *Aspergillus* infections, or subacutely, as in cryptococcal meningitis [6, 24, 28, 32, 36–41]. The clinical presentation of different CNS infections is not specific.

Brain imaging helps to classify CNS infections into two main entities: those which present predominantly as focal lesions (brain abscess) and those presenting usually as meningitis or meningoencephalitis, as shown in the Table 18-1. MRI appeared to be more sensitive than CT in identifying the brain lesions.

18.2 Focal Lesions

18.2.1 Incidence

Brain abscesses may develop in 5 % of HSCT recipients, 0.3–0.6 % in liver; 0.2–0.4 % in kidney; 1.3 % lung; and in about 1 % in heart and heart–lung recipients [24, 37, 42].

18.2.1.1 Etiology

Fungi are the main pathogen causing brain abscess in all transplant recipients. Among 58 HSCT recipients with brain abscess a fungus was isolated in 92 % of cases [36]. *Aspergillus* species were the most prevalent fungi (58–65 %) [36, 37]. *Candida* species were reported in some series [18, 36], although other studies did not recognize it at all [24, 28, 37]. Other fungal pathogens include *Cladophialophura bantiana*, *Scedosporium apiospermium*, *Microascus cinereus*, *Rhizopus*, *Absidia*, *Scopulariopsis,* and *Pseudallescheria* species [36, 37]. Among the *Aspergillus* species, *Aspergillus fumigatus* is the most frequent [24, 32, 43, 44]. The fungal pathogens causing focal brain lesions are described in Table 18-2.

TABLE 18-2. Fungal pathogens which cause focal brain lesions in transplant patients

Hyalohyphomycosis [45, 46]	Mucormycosis [47, 48]	Phaeohyphomycosis [46, 49–51]
Aspergillus fumigatus	*Rhizopus*	*Cladophialophora bantiana*
Aspergillus flavus	*Mucor*	*Scedosporium prolificans*
Aspergillus terreus	*Rhizomucor*	*Exophiala jeanselmei,and E. dermatitidis*
Aspergillus niger	*Absidia*	*Pyrenochaeta romeroi*
Scedosporium apiospermum (*Pseudallescheria boydii*)	*Cunnighamella*	*Cladosporium species*
	Apophysomises	*Ochroconis gallopavum*
		Ramichloridium mackenziei
		Chaetomium strumarium and C.atrobrunneumy
		Scopulariopsis brumptii
		Fonsecaea pedrosoi
		Microascus cinereus

18.2.1.2 Diagnostic Criteria for CNS Invasive Fungal Disease (IFD) [52]

Proven CNS IFD is diagnosed when there is one of the following:

1. Microscopic analysis of brain tissue: histopathologic, cytopathologic, or direct microscopic examination of a brain tissue in which hyphae, melanized yeast-like forms or yeast cells are seen accompanied by evidence of associated tissue damage.
2. Culture of CSF or of brain tissue obtained from a radiologically abnormal site consistent with an infectious disease process, with recovery of a mold, "black yeast" or yeast.
3. Cryptococcal antigen in CSF.

Probable CNS IFD in transplant recipients requires a clinical criterion and a mycological criterion. Cases that meet the clinical criteria but not the mycological criteria are considered as **possible CNS IFD**.

Clinical criteria for CNS infection include one of the following two signs: focal lesions on imaging or meningeal enhancement on MRI or CT.

Mycological criteria for probable IFD include:

1. Direct test (cytology, direct microscopy, or culture) of mold in bronchoalveolar lavage fluid, bronchial brush, bronchial biopsy, or sinus aspirate samples, indicated by one of the following:
   - Presence of fungal elements indicating a mold
   - Recovery by culture of a mold
2. Indirect tests (detection of antigen or cell-wall constituents):
   - For aspergillosis—galactomannan (GM) antigen detected in plasma, serum, or CSF
   - For IFD other than cryptococcosis and mucormycosis—b-D-glucan detected in serum

18.2.1.3 Treatment

Generally, treatment modalities include drug therapy and surgical intervention. The drug therapy for each pathogen will be discussed later. In a retrospective analysis of 192 proven or provable fungal CNS infections *Aspergillus* spp. (63 %) and *Scedosporium* spp. (18 %), success rate in patients treated with voriconazole was 14 % in HSCT, 54 % in hematological malignancy patients, 40 % in SOT, 45 % in patients with other chronic immunosuppression, and 72 % in patients with other conditions ($p<0.001$). In this study, combination antifungal therapy showed a trend toward an improved response rate ($p=0.09$) and superior survival ($p=0.0149$), while patients receiving neurosurgical interventions showed superior responses ($p=0.0174$) and survival ($p=0.0399$). In all, 49 % of patients died, 71 % (67/94) due to fungal infection. The overall median survival was 297 days (range 3 to >2000). Pediatric ($p=0.014$) patients exhibited superior survival compared with adults [53].

18.2.2 *Aspergillus*

18.2.2.1 The Pathogen

The pathogen is described in Chap. 40, Mold Infections after hematopoietic stem cell transplantation, and Chap. 41, *Aspergillus* and other mold infections after SOT.

18.2.2.2 CNS Involvement

The primary site of infection with *Aspergillus* is the respiratory tract, and it can disseminate, involving mainly the CNS [54]. Dissemination to CNS was found in 16 % of SOT and HSCT recipients with pulmonary aspergillosis [55, 56]. Once *Aspergillus sp*. spread beyond the lung it can be found in very high proportion in brain of fatal cases, for example,

50 % in one study in HSCT patients, when examined both macroscopically and microscopically [57, 58]. In a retrospective analysis of 322 patients undergoing allogeneic HSCT, 55 % of patients with pulmonary disease developed CNS involvement [43]. Lungs were involved in the majority of cases with CNS aspergillosis [24, 58, 59], and only 10 % have isolated CNS involvement [32]. *Aspergillus* sinusitis is not frequent (8 %) in patients with CNS aspergillosis [60]. In SOT patients, dissemination was much more frequent in liver transplant recipients, 46–60 %, compared to 22 % in lung, 17 % in heart, and 10 % in renal transplantation (10 %) [31, 55, 60]. However, one study showed that in the current era of liver transplantation, invasive aspergillosis (IA) occurs later in the posttransplantation period, is less likely to be associated with CNS infection, and is associated with a lower mortality rate, compared with IA in the early 1990s [44].

18.2.2.3 Incidence

In HSCT recipients, 3 % developed *Aspergillus* brain abscess [39, 43]. In SOT recipients, incidence is less than 1 % [24, 37, 42].

18.2.2.4 Time After the Transplantation

Median time to the diagnosis of CNS aspergillosis was 110–124 days (range 18–395 days) post-HSCT [6, 37, 39].

Median time of development of CNS aspergillosis in SOT varies in different studies from 15 (range, 8–81 days) to 538 days (range 14–1260 days) [32, 37, 60]. There are two peaks of infection. The first one is during the early post-transplantation period. In one study in early 1990s in SOT recipients, 64 % of CNS aspergillosis developed in the initial 2 months after transplantation [32]. In the 1990s, several studies showed that liver transplant recipients tend to develop CNS aspergillosis earlier than other transplant patients (50–73 % of cases developed in the first month after transplantation [24, 32, 60]). In the current era, one study in liver transplantation showed that IA occurs later in the post-transplantation period [44]. Chronic rejection and/or retransplantation during a period of intense immunosuppression are associated with a second peak of incidence, after the first year [32]. In one study in heart transplant patients, CNS involvement was more common in patients with late aspergillosis (>3 months after transplant) as compared to early disease [61]

18.2.2.5 Risk factors

The risk factors for IA infections in HSCT and SOT can be divided to patient related, transplant related and environmental factors (see session???).

Table 18-3. Clinical presentation of CNS Aspergillosis [6, 24, 32, 36, 37, 39]

Clinical signs/symptoms	Percentage of patients with sign or symptoms
Fever (>38.0)	45–76 %
Altered mental status	35–82 %
Hemiplegia/paresis	27–50 %
Cranial nerve abnormalities	29 %
Seizures	21–40 %
Nausea/vomiting	18 %
Headache	12–60 %
Hemianopia	Reported
Meningismus	Reported

18.2.2.6 Clinical Manifestations

The common clinical manifestations of CNS aspergillosis, which are similar in SOT and HSCT recipients [37], are summarized in Table 18-3. The most common clinical manifestations of CNS aspergillosis are focal neurologic deficits and seizures, due to stroke or mass effect [62]. The neurological symptoms usually progressed quickly [32, 39].

18.2.2.7 Laboratory

The most common finding in CSF is a moderate increase in the protein concentration [39]; however, CSF findings are often nonspecific [62]. Laboratory diagnosis of IA includes blood testing of GM, PCR, and (1-3)-β-D-Glucan (BDG). In HSCT recipients with pulmonary IA serial GM blood test shows sensitivity of 50–90 %, specificity 89–98 %, PPV 46–90 %, and NPV 94–98 % [63–66]. There is also good correlation between GM and outcome from IA [67]. Serum GM measurement is less studied in SOT patients and shows lower sensitivity results for the diagnosis of pulmonary aspergillosis: 56 % in liver [68], 30 % in lung transplant recipients [69]. Blood PCR was used for the non-culture diagnosis of IA in HSCT recipients, showing variable sensitivity (63.6–100 %) and specificity 63.5–100 % [70–73]. Data on GM, BDG, or PCR in CSF are limited [74–77], but it may be positive in CNS aspergillosis, while they were found negative in small number of CSF samples of patients having CNS disease of other etiology [75]. The sensitivity and specificity of Platelia ELISA for the detection of galactomannan in CSF were 80 % and 100 %, respectively, in a study describing five patients with proven CNS aspergillosis [76]. Culture of CSF samples yielded negative results, but PCR yielded positive results for all patients. Galactomannan levels in CSF were significantly higher than in serum [77]; decreasing galactomannan CSF levels correlated with clinical improvement [74].

18.2.2.8 Imaging

CT and especially MRI are the imagings that detect easily the focal lesions suggestive of CNS aspergillosis [37, 59, 78]. Characteristically they show infarction and necrosis, some with hemorrhage, due to the angioinvasive nature of the fungus [39, 59, 79, 80]. Lesions of CNS aspergillosis are multiple in about 70 % of cases [24, 37, 39], with a mean of 2.8 lesions per patient (range, 1–8) [37]. The most common location for lesions is the frontoparietal region (54–82 %), followed by the basal ganglia or thalami (17–72 %), occipital lobe (13 %), temporal lobe (9 %), and cerebellum (7 %) [24, 37, 80]. Corticomedullary junction involvement is common [78, 81]. In one series, the corpus callosum was involved in 7/18 (40 %) patients [80]. As the corpus callosum is not commonly subject to pyogenic infection and thromboembolic infarction, callosal lesions strongly suggest the diagnosis of aspergillosis [78].

Progression of the size and number of lesions during the first 10 days of therapy was reported in one series, in all their 11 patients who underwent serial imaging studies [80]. Serial imaging performed in the patient who recovered showed regression of small lesions and calcifications in the large lesions [80].

18.2.2.9 Treatment and Outcome

In one series, death because of CNS aspergillosis contributed 8 % of all causes of death during the first year post-HSCT during 1989–2000 [39].

Mortality in CNS aspergillosis in HSCT and SOT patients treated with amphotericin B or its lipid formulations with or without combination with itraconazole or 5-fluorocytosine (5-FC) was 94–100 % [6, 37, 39, 55, 82, 83]. During 1989–2000, one series reported on median survival of only 7 days (range 0–27 days) from the onset of the neurological symptoms or signs, in 14 HSCT recipients suffering from CNS aspergillosis and treated with amphotericin B [39].

Management of CNS fungal infection is complicated by the poor CNS penetration of a lot of agents. Drugs with acceptable CSF penetration include 5-FC (drug penetration 74 % serum concentration), fluconazole (60 % serum concentration), and voriconazole (50 % serum concentration). Other antifungals with poor CSF penetration are amphotericin B dexochycolate (<4 % serum concentration), itraconazole (<5 % serum concentration) [84]. Caspofungin administration reveals brain tissue/plasma ratio of 0.2 in animal studies [85]. Data on posaconazole CSF concentration levels show conflicting results [86–88]. Voriconazole penetrates the CSF and brain tissue of animals and the penetration is not dependent on meningeal inflammation. In humans, 1–10 h after receipt of voriconazole, the CSF concentrations ranged from 0.08 to 3.93 mg/mL, and the ratio of CSF to plasma concentration ranged from 0.22 to 1.0 (median, 0.46). The moderate lipophilicity and large volume distribution of voriconazole may well contribute to the higher concentrations detected in brain tissue, compared with those detected in the CSF or blood [55, 89].

Initial therapy of IA, including those with CNS involvement, with voriconazole lead to better responses and improved survival—of 27 % (SOT) and 22 % (HSCT) in CNS aspergillosis [90], in another study 16 % showed partial response and 26 % showed stabilization with CNS aspergillosis [91] and also resulted in fewer severe side effects than with amphotericin B [92]. Neurosurgical intervention is associated with improved survival [90, 91, 93, 94]. Thus, voriconazole is currently the drug of choice for CNS aspergillosis. Although there are no formal guidelines regarding therapeutic drug monitoring in CNS aspergillosis, maintaining trough concentrations of 2–5 μg per milliliter in serum is recommended [95]. Itraconazole, posaconazole, or LFAB are recommended for patients who are intolerant or refractory to voriconazole [96]. Caspofungin is an alternative treatment. In one small series of proven or possible CNS aspergillosis, including those with disseminated infection, 2/6 of the patients had a favorable response with caspofungin therapy [97].

Local intracavitary treatment with amphotericin B in adjunction to systemic amphotericin B and surgical therapy was described in case reports in transplant patients [98].

Combination therapy may have benefit in the treatment of CNS aspergillosis. In an immune suppressed murine model, significant enhancement of efficacy for the prolongation of survival was attained with the ambizome-plus-voriconazole regimen as compared to each agent given alone ($p = 0.0001$) [99]. Combination therapy with voriconazole and caspofungin for IA showed a trend to lower mortality in transplant patients, although only minority suffered from CNS disease [100, 101]. Treatment of IA with the combination of voriconazole and anidulafungin was associated with a nonsignificant but clinically meaningful survival benefit in patients with HM or HSCT in a recent randomized double-blind, placebo-controlled multicenter trial [102].

Monitoring includes serial imaging every 1–2 weeks until stabilization of patient's condition [95].

18.2.3 Emerging Fungal Pathogens

Infections due to infrequently encountered fungi (e.g., dematiaceous fungi and zygomycetes) have become increasingly common in immunocompromised hosts. Non-*Aspergillus* molds were reported in 35–45 % of fungal brain abscesses in transplant patients [24, 37].

Transplant patients suffering from invasive infection due to non-*Aspergillus* molds (zygomycetes, phaeohyphomycetes, and hyalohyphomycetes) vs. *Aspergillus* have more frequent CNS involvement, 23 % vs. 5 % in one study in liver and heart transplant recipients. In this study, infections

limited to the CNS were not observed with infections due to *Aspergillus* species, as compared with 21.4 % with other mycelial fungi ($p=0.017$) [49].

18.2.4 *Scedosporium apiospermum*

18.2.4.1 Pathogen

S. apiospermum (sexual form, *Pseudallescheria boydii*) is an emerging pathogen which belongs to hyalohyphomycosis.

18.2.4.2 Incidence

The incidence of invasive *Scedosporium apiosrermium* infections was 0.2 % among 5589 HSCT patients [103]. In a series of 23 SOT patients with *Scedosporium apiospermum* infections, 11 had CNS disease, with overall incidence of 1 per 1000 patients and CNS involvement of 0.5 per 1000. There was a trend of higher incidence in patients receiving lung transplants compared with other transplant organs ($p=0.06$) [45].

18.2.4.3 Time After Transplantation

In HSCT, invasive infections with *Scedosporium* species occurred mainly in the first 30 days after transplantation [103]. In SOT, it was diagnosed at a median of 4 months (range, 0.4–156 months) after the transplantation [45]. In cystic fibrosis (CF) patients after lung transplantation, the median time from transplantation to the onset of infection was shorter—5 weeks (range, 2 weeks to 7.5 months), possibly explained by the colonization with *Scedosporium*/*Pseudallescheria* at the time of transplantation [104].

18.2.4.4 Clinical Manifestation

CNS involvement was mainly brain abscess ($n=10$) and occasionally meningitis ($n=1$). Half patients with brain abscess had a single brain lesion and the other half had multiple lesions [45].

18.2.4.5 Treatment and Outcome

Voriconazole is the current recommended therapeutic agent, with 43 % (9/21 patients) successful response in CNS infection in one report [46]. In another report, all four CF lung transplant patients with CNS *Scedosporium* infection died [104]. Combination of voriconazole and surgical drainage may be needed [45].

18.2.5 Phaeohyphomycosis

Agents of phaeohyphomycosis, which are called also dematiaceous fungi, are increasingly recognized as a cause of serious disease in patients with immunodeficiency. Most agents of phaeohyphomycosis are found in soil and are ubiquitous in the environment. These fungi have a high degree of neurotropism. Animal model showed that cortisone-treated mice intranasally inoculated with *Cladophialophura bantiana*, develop CNS disease following hematogenous dissemination from a primary pulmonary focus [105]

18.2.5.1 Pathogen

The characteristic feature of all these species is the presence of melanin in their cell walls, which imparts the dark color to their conidia or spores and hyphae [106]. Among 18 transplant patients with CNS phaeohyphomycosis, *Cladophialophura* was isolated in seven (40 %, five of them *C. bantiana*), four *Ochroconis gallopavum*; *Ramichloridium mackenzei* and *Chaetomium strumarium* (two each) and *Chaetomium atrobrunneum*, *Scopulariopsis brumptii* and *Fonsecaea pedrosoi* (one each) [50].

18.2.5.2 Incidence

CNS phaeohyphomycosis is a rare infection, which occurs mainly in immunocompetent young males but may occur also after transplantation [50]. In one series, the incidence of phaeohyphomycosis infection varied from 0.16 % among liver transplant patients to 3.6 % among kidney/pancreas transplant patients, with an overall incidence of 0.7 % [107]. Interestingly, male predominance is mentioned in all the series: 64–83 %, probably because of occupational or environmental exposure [45, 46, 50]. Only 1.8 % of 56 phaeohyphomycosis cases were in CNS [108].

18.2.5.3 Time After Transplantation

Infection with phaeohyphomycosis can appear 2–128 months after SOT [49, 107]. Median time was 20 months in one series [107]; it was 358 days in summary of 56 cases with proven and probable phaeohyphomycosis in transplant patients from the Transplant Associated Infection Surveillance Network (TRANSNET) (HSCT 100 days; SOT 685 days; $p=<0.001$) [108]. Median time after transplantation was 11–17 months for *Ochroconis gallopava* (range 2–44 months) [51, 109].

18.2.5.4 Clinical Presentation

Thirty to fifty percent of transplant patients with *Ochroconis gallopavum* infection had CNS involvement [51, 109].

All except one of 101 cases of CNS phaeohyphomycosis presented with brain abscess, usually single. Clinical signs on presentation included headache 59 %, neurological deficits 54 %, seizures 34 %, altered mental status 32 %, fever 32 %, and nausea and vomiting 19 %. Clinical presentations varied considerably, although one neuro-

logic sign or symptom was present in all cases. However, the classic triad of fever, headache, and focal neurologic deficits described in patients with brain abscess was rarely observed [50].

18.2.5.5 Treatment and Outcome

In one series, 4/6 transplant patients with *Ochroconis gallopavum* CNS infection died [51]. In another report, 12/18 (67 %) transplanted recipients with brain abscess caused by phaeohyphomycosis died [50]. The most frequently used drug was amphotericin B; voriconazole was not used at all [50]. In this review, combination of amphotericin B, 5-FC, and itraconazole was associated with improved survival, although there were relatively few cases in which this triple combination was used [50]. Overall mortality in transplant patients with phaeohyphomycosis was 25 % and was higher in HSCT than in SOT (42 % vs. 10 %; $p = <0.001$) [108]. In another summary, voriconazole (44.6 %) and amphotericin B preparations (37.5 %) were the most common antifungal therapies in patients with phaeohyphomycosis [108]. Itraconazole and voriconazole demonstrate the most consistent in vitro activity against phaeohyphomycosis, except *S. prolificans*, which is resistant to all azoles [106]. Complete excision of brain abscesses improves the outcome [50]. Amphotericin B may be used for severe infections in unstable patients; high doses of lipid formulations may have a role in the treatment of refractory cases or in patients intolerant of standard amphotericin B. Combination antifungal therapy with voriconazole and a lipid formulation of amphotericin B, pending the availability of in vitro susceptibility data, is recommended in conjunction with surgery [95]. Once the infection is under control, longer term therapy with a broad spectrum oral azole is a reasonable approach, until complete response is achieved [106].

18.2.6 Mucormycosis

18.2.6.1 Pathogen

Mucormycosis is an invasive mold infection with high morbidity and mortality (see Chaps. 40 and 41). Portals of entry include sinuses, lungs, gastrointestinal tract, and skin.

18.2.6.2 Incidence

Mucormycosis represents 2 and 8 % of invasive fungal infections in SOT and HSCT recipients [30].

18.2.6.3 Time After Transplantation

Mucormycosis may occur as early as 1 day and as late as several years after HSCT and SOT [47, 49].

18.2.6.4 Clinical Manifestation

Among HSCT recipients with mucormycosis, 10–15 % have involvement of the CNS, most frequently in the context of dissemination. Among 116 SOT patients with mucormycosis, in 19 % CNS was involved as a part of rhinocerebral or disseminated disease [47]. A half of patients after kidney transplantation with mucormycosis had rhinocerebral disease [110]. The initial symptoms of rhinocerebral mucormycosis are consistent with either sinusitis or periorbital cellulitis. The infection may rapidly extend into the neighboring tissues. Extension from the ethmoid sinus through the lamina papyracea involves the orbit, extraocular muscles, and optic nerve; extension from the maxillary sinus into the oral cavity may present as a painful black necrotic ulceration on the hard palate [111]. Among 90 SOT recipients with rhino-orbital-cerebral mucormycosis, the maxillary cavities and brain were involved in 80 % and 57 %, respectively [112]. Cranial nerve may be involved, including vision loss. A bloody nasal discharge may be the first sign that infection has invaded through the turbinates into the brain. When there is extensive CNS involvement, the angioinvasive nature of the fungus may result in cavernous sinus thrombosis and internal carotid artery encasement and thrombosis with extensive resulting cerebral infarctions [113]. Diplopia and ophthalmoplegia may be the earliest manifestations of cavernous sinus syndrome [111].

18.2.6.5 Treatment and Outcome

Patients with CNS mucormycosis had a very grave prognosis, with mortality above 90 %, even when combined amphotericin and surgery approach was applied in one study [47]. In another study, mortality however was 30.8 % in kidney SOT [110]. Doses of 10 mg/kg/day of liposomal amphotericin are suggested for infections involving the CNS [114]. For salvage treatment who need prolonged continuation or maintenance therapy posaconazole 4 × 200 mg/day is recommended [114, 115].

One study described 24 patients refractory or intolerant to previous therapy, the majority post-HSCT or SOT, 58 % with CNS involvement as a part of rhinocerebral or disseminated disease. Under treatment with posaconazole, 79 % achieved complete or partial response. Surgery, in addition to posaconazole, improved the prognosis 5.7 times [48]. Success was achieved in 73 % of patients with CNS mucormycosis in a retrospective summary of 91 patients (40 % transplant recipients) refractory or intolerant to prior antifungal treatment who participated in the compassionate-use posaconazole program [116]. Surgical debridement is recommended, it was associated with improved outcome in patients with rhino-orbito-cerebral mucormycosis [112, 114]. The use of a combination of a polyene and an echinocandin may be an option in salvage therapy after failure of appropriate first-line therapy [114]. Salvage therapy with isavuconazole was

reported [117]. Therapeutic drug monitoring is recommended where possible [114]. Using granulocyte colony-stimulating factor in patients with ongoing neutropenia is recommended [114, 115]. The duration of antifungal treatment should be determined on an individual basis, but therapy usually continues for at least 6–8 weeks [114]. Routine use of adjunctive iron chelator and hyperbaric oxygen therapy is not recommended [114].

18.2.7 *Candida*

Although *Candida* was reported in the past as causing CNS abscesses or meningoencephalitis [6, 10, 17, 34, 36], it is currently not considered as a significant pathogen for CNS disease [24, 28, 37].

18.2.8 *Nocardia*

18.2.8.1 Pathogen

The genus *Nocardia* consists of Gram-positive, variably acid fast, filamentous, branched cells [118]. *N. asteroides* is the most common species identified in nocardial brain abscesses [119]. Nocardial lesions frequently erode into blood vessels, causing dissemination.

18.2.8.2 Incidence

The incidence of invasive nocardiosis was 0.3–1.7 % in allogeneic and 0.2 % in autologous HSCT recipients [120, 121]. Organ transplantation is one of the predisposing conditions for nocardial infection, being reported as a risk factor in 22 % of patients with nocardiosis [118]. The incidence was 0.04–0.7 % of renal [122], 0.65–2.5 % heart [34], and 0.6–3.5 % of lung transplant recipients [123, 124]. Decrease in the incidence of nocardiosis has been observed [124, 125].

In a summary of 1050 patients, immunocompetent and immunocompromised, with systemic nocardiosis, CNS involvement was reported in 44 % [118]. CNS disease is the most common extrapulmonary site of disease, it occurred in 3/35 (9 %) SOT recipients and in 3/10 (30 %) of hematological patients with nocardiosis [123, 126]. Summary of 66 cases with nocardiosis in kidney transplant patients reported in the literature during 30 years revealed cerebral involvement in 27 cases (41 %), and in 75 % of disseminated infection [125].

18.2.8.3 Time After Transplantation

In the study on SOT patients, in 63 % nocardiosis occurred within 1 year after transplantation, and in 14 % >5 years after transplantation [123]. Infection in the first month after transplantation is rare, median time varied 1.25–48 months after SOT (34 months for kidney transplant patients [125]) and 6–10 months after HSCT [124].

18.2.8.4 Risk Factors

In HSCT, almost all infections occurred in allogeneic HSCT vs. autologous and GVHD was present in almost all patients [120, 121]. Eighty percent of hematological patients with nocardiosis were lymphopenic, and the same proportion were treated with steroids [126]. Four of six patients in one study had extensive exposure to soil or dust before nocardiosis developed [121]. Receipt of high-dose prednisone in preceding 6 months, ($p=0.003$), elevated median calcineurin inhibitor level in preceding 30 days ($p=0.012$) and CMV disease ($p=0.047$) were identified as risk factors in SOT recipients [123].

18.2.8.5 Clinical Manifestations

The clinical presentation of CNS infection by *Nocardia* spp. may be acute with rapid progression, but can be also insidious in onset [118]. Some patients present with focal deficits, headache or confusion, seizures, and psychological abnormalities [119, 125]; others have no fever or neurological signs at all [118]. Therefore, CNS imaging is advised in transplanted patient with the evidence of *Nocardia* infection [127].

18.2.8.6 Imaging

The most common clinical CNS manifestation is single or multiple (38 %) abscesses affecting any part of the brain. Radiological evidence of meningitis may be present [118, 119, 127].

18.2.8.7 Treatment and Outcome

Overall, 64 % of immunocompromised patients, 40 % of whom were transplanted patients, died of nocardial brain abscess. The mortality rates for single and multiple abscesses were 33 and 66 %, respectively ($p<0.0003$) [119]. Prophylaxis with TMP-SMZ, administered to prevent *Pneumocystis jirovecii* pneumonia, does not prevent nocardial infections [120, 121, 123]. Therapy of choice for nocardial brain abscess is TMP-SMX. Other treatment options include minocycline, imipenem, or a third-generation cephalosporin, like ceftriaxone [119, 123].

Three-drug regimen comprising trimethoprim-sulfamethoxazole, amikacin, and either ceftriaxone or imipenem can be initiated until susceptibility results available [124, 128]. Linezolid may be used due to high CNS penetration, evidence of high success rate (80 %), no resistance reported and oral availability [124, 129]

Regardless of the antibiotic regimen selected, it should be administered intravenously for at least 6 weeks, depending on clinical response, followed by oral antibiotics for at least 1 year [119].

Analysis based on 131 cases of nocardial brain abscess (13 % of them in transplant recipients) suggested that all abscesses larger than 2.5 cm should be aspirated, regardless of the immune status of the patient [119]. Contrast-enhanced CT scans or MRI should be obtained every 2 weeks or after any clinical deterioration. If any abscess enlarges after 2 weeks of antibiotic therapy or fails to shrink after 4 weeks of therapy, a craniotomy should be performed to excise the abscess. If the abscess is surgically inaccessible, aspiration/drainage may be repeated, although the likelihood of success is reduced [119]. Small abscesses may resolve with long-term medical treatment.

18.2.9 *Toxoplasma*

18.2.9.1 Pathogen

Toxoplasma gondii is an intracellular protozoan parasite [130]. *Toxoplasma* encephalitis occurs mainly from reactivation of latent microorganisms in seropositive recipients, but may be due to new infection.

18.2.9.2 Incidence

The incidences of toxoplasmosis after transplantations are detailed in Chaps. 43 and 44.

18.2.9.3 Time After Transplantation

Cerebral toxoplasmosis occurred from day 4 to day 689 after HSCT (median 64–84 days [6, 130–133]). The disease usually begins early after HSCT, 30 % within the first 30 days, 67–100 % developing in the first 100 days [6, 130, 132, 134, 135] and 95 % within 6 months after the HSCT [130, 136]. Toxoplasmosis was diagnosed within the first 6 months after transplantation in 64 % of SOT patients [137]. Among 64 noncardiac SOT recipients who developed toxoplasmosis, 65 % developed symptoms within the first month and 86 % within 3 months after the transplantation [138]. In high-risk patients (donor positive/recipient negative), toxoplasmosis developed earlier than in the others [138].

18.2.9.4 Clinical Manifestations

Neurological involvement is frequent in toxoplasmosis in HSCT recipients, with 66–80 % of patients presenting with neurological symptoms [134]. In HSCT who developed toxoplasmosis, brain or brain stem was involved in 74–89 % of patients, and in 94 % of autopsies [130, 131]. Isolated CNS involvement occurred in 44–50 % of patients; in another 25–56 % CNS was involved as a part of disseminated disease [131, 139]. Review of 53 *Toxoplasma* cases in noncardiac SOT recipients revealed a different picture: neurologic manifestations at onset were only in 26 % of patients; only 5.5 % had isolated CNS disease [138].

Among 22 SOT recipients, 22.7 % presented with brain abscess and 4.5 % with meningitis [137]. Presenting symptoms include seizures 45 %; neuropsychological signs, including somnolence and obtundation 35–50 %; focal signs, as hemiparesis, hemianopia, ataxia, dysarthria, 25 %; headaches 25 %; coma 20 % [6, 130]. High grade fever (>39 °C) is frequent (80–91 %) [130, 132], although in another series it was reported in 43 % of HSCT recipients [131]. Relapse was reported in 30 % of HSCT patients, 33–318 days after the first episode [135]. Concommitant severe infections were diagnosed in 27–40 % [130, 131, 137].

18.2.9.5 Diagnostic Criteria

Definite *Toxoplasma* disease is determined by histological or cytological demonstration of tachyzoites or isolation of the parasite by culture in brain tissue samples obtained by biopsy or at autopsy.

Probable *Toxoplasma* disease is determined by clinical and radiologic evidence suggestive of CNS involvement plus at least one positive PCR test from blood, CSF, or BAL.

Possible *Toxoplasma* disease is determined by CT or MRI highly suggestive of CNS toxoplasmosis (as considered by each hospital's neuroradiologists) and response to anti-*Toxoplasma* therapy and absence of another pathogen that may explain the findings [130].

18.2.9.6 Laboratory

Blood PCR can be used for the diagnosis of patients with CNS toxoplasmosis, although the rate of positive blood PCR was much lower in cerebral disease (13 %) vs. disseminated (78 %) disease in SOT and HSCT and other immunocompromised patients [140]. Routine PCR testing of peripheral blood specimens may be an appropriate tool for guiding preemptive therapy in patients at very high risk of developing invasive disease. The incidence of disease was 38 % in patients with positive *Toxoplasma* PCR in blood drawn prospectively during follow-up after 110 HSCT recipients, whereas it was 0 % in patients with negative PCR ($p < 0.0001$). Copy number of *Toxoplasma* ribosomal DNA in blood samples was higher in patients with invasive disease than in patients with *Toxoplasma* infection [135].

CSF PCR is positive in some patients [133, 141, 142]. The value of CSF PCR was checked in HIV-infected patients and showed the sensitivity, specificity, and positive and negative predictive values for detecting *T. gondii*

62.5 %, 100 %, 100 %, and 85.7 %, respectively [143]. The negative predictive value of PCR results from blood might also be dependent on the timing and the repetition of blood sampling [144].

CSF protein level may be slightly elevated, otherwise CSF was normal [133].

A seroconversion occurring early after transplant, with demonstration of IgM and IgG antibodies, and eventually IgA and IgE, is a strong indication of an acquired (and probably transmitted) infection with subsequent risk of disease. In some patients, positive IGM was early indicator of acute infection [145]. The majority of SOT patients with toxoplasmosis were diagnosed due to seroconversion [137]. However, the serological diagnosis of toxoplasmosis has important limitations in transplant patients, as the underlying immunosuppression alters antibody production and its kinetics [144].

In cases of profound immunosuppression, the antibody response might be lacking or atypical [139]. A reactivation should be suspected upon observation of a rise in specific antibody titers, usually IgG antibodies without IgM or IgA antibodies [144].

18.2.9.7 Imaging

Multiple lesions in the basal ganglia and subcortically in supra- and infratentorial location are the most frequent imaging finding in HSCT patients [6, 133, 136]. Hemorrhage is more frequent finding in HSCT patients vs. others due to low platelet count [6, 136].

There are two patterns of MRI imaging in HSCT patients with CNS toxoplasmosis. Disease which manifests early (in the first 3 months) posttransplant is characterized by minimal space-occupying effects and enhancement. Oppositely, late disease shows typical MRI appearance of *Toxoplasma* encephalitis with multiple lesions and ring enhancement with perifocal edema, reflecting higher ability to cause inflammation.

18.2.9.8 Treatment and Outcome

High suspicion for toxoplasmosis is needed for every patient with unexplained neurological findings [134]. Presence of concomitant infection with overlapping clinical presentation and false negative laboratory tests makes diagnosis challenging in some cases [146]. The recommended treatment is pyrimethamine, sulfadiazine plus folinic acid [138], followed by suppressive doses of TMP-SMZ or an alternate regimen, as written below, for the duration of their immunosuppression [147].

The overall mortality from *Toxoplasma* encephalitis was 55–90 % in HSCT [6, 134], and 35 % in SOT patients [138]. Good prognostic factors include: cerebral disease vs. disseminated (42 % vs. 80 % mortality) because CNS toxoplasmosis is usually diagnosed earlier (80 % during life vs. 20 % in disseminated infection) and treated appropriately [131]; early diagnosis and treatment with anti-*Toxoplasma* antibiotics, especially with reduction of immunosuppression improved survival [130, 138]; toxoplasmosis that was discovered late (36 % mortality) after transplantation, vs. early (70 % mortality) [6, 130].

18.2.10 Posttransplantation Lymphoproliferative Disorder (PTLD)

PTLD is a serious complication of transplantation. 12–22 % of PTLD involve CNS [148–150].

18.2.10.1 Incidence of CNS PTLD

Retrospective analysis of the complete autopsy records and clinical histories of 500 adults who underwent SOT showed PTLD involving the brain in 2 % of the liver, 3 % of the heart, and 7 % of the heart–lung recipients [35]. Among 910 PTLD cases from the USA reported to the Israel Penn International Transplant Tumor Registry, CNS was involved in 136 (15 %) cases, 15 occurred in pediatric patients. Isolated CNS involvement occurred in almost half of patients in several series. By organ transplanted, the highest incidence of CNS involvement occurred in pancreas transplant recipients (27 %), followed by those with kidney (18 %), heart (13 %), liver (11 %), and lung transplants (13 %) [148, 150]. Others reported on increased frequency of CNS PTLD in kidney transplant patients 55–78 % of all PTLD cases [151, 152].

The 3-year cumulative incidence of EBV-associated CNS disease was 8.6 +/− 2.4 % in allogeneic [153] and 4.3 % in cord blood HSCT patients [154].

18.2.10.2 Time After Transplantation

PTLD occurs late after SOT (see Chap. 26). The median time to onset was 19–33 months (range, 3–131 months) in a summary on 45 patients, predominantly adult kidney transplants recipients with isolated CNS PTLD [148, 149, 155, 156]. Among 84 CNS PTLD patients in a multicenter, international analysis, median time of SOT-to-PTLD was 54 months, 83 % of PTLDs were late onset (>1 year post-SOT); among them 36 % occurred >10 years after SOT [151].

PTLD may appear earlier in HSCT patients. The median time to PTLD was 73 (36–812) days after cord blood [154] and to CNS manifestation onset ~48 (22–184) days post-allogeneic transplantation [153, 157].

18.2.10.3 Risk Factors

Several risk factors for developing PTLD have been identified (see Chap. 26 Epstein–Barr Virus Infection and Lymphoproliferative Disorders after Transplantation).

18.2.10.4 Clinical Manifestations

The main symptom, encountered in 75–84 % was focal deficits, such as hemiparesis or aphasia. This was the isolated manifestation in about a third of patients, or associated with seizures in another third or symptoms of increased intracranial pressure in 13–36 %. Mental changes occurred in 8–50 % [149, 155]. Fever was frequent [153]. The mean duration of symptoms before diagnosis was 36 days (range 5–98 days) [149, 155]. Nonspecific symptoms of headache and altered mental status may be the only complaint [158].

18.2.10.5 Diagnostic Criteria

Definitive diagnosis of PTLD is based on symptoms, signs, and imaging consistent with PTLD together with at least two and ideally three of the following histological features: (1) disruption of underlying cellular architecture by a lymphoproliferative process; (2) presence of monoclonal or oligoclonal cell populations as revealed by cellular and/or viral markers; (3) evidence of EBV infection in many of the cells, that is, DNA, RNA, or protein. Definitive diagnosis of EBV-PTLD requires biopsy and histological examination (including immunohistochemistry or flow cytometry for CD19+ and CD20+). Detection of EBV nucleic acid in blood is not sufficient for the diagnosis of EBV-related PTLD [149, 155, 159, 160].

Positive CSF EBV PCR should be interpreted with caution, and other treatable etiologies have to be excluded. In one study, in 15/32 (47 %) of the patients with positive EBV DNA in CSF (18 were transplant patients) the clinician had a strong suspicion of cause other than EBV for the patients' CNS symptoms/findings, in some of them alternative diagnosis was confirmed [161].

18.2.10.6 Laboratory

The most common finding is an increased protein concentration while pleocytosis in CSF was found in some patients. EBV PCR in CSF may be negative [149, 155]. The laboratory findings used to identify EBV infection in immunocompetent persons (atypical lymphocytosis, VCA-IgM, and heterophile antibodies) may or may not be present in immunosuppressed patients. The absence or loss of anti-EBNA may precede PTLD, and the lack of seroconversion following primary EBV infection may be a risk factor for mortality from PTLD [159]

Regular monitoring of EBV DNA blood levels in allogeneic HSCT patients revealed that DNA loads of blood increased 3–14 days before the clinical manifestations of EBV-associated PTLD. The EBV DNA loads of CSF were higher than that of blood in patients with EBV-associated CNS PTLD. Dynamics of CSF EBV DNA levels correlated with the disease course—they declined with the control of diseases, and increased in patients who did not respond to treatment [153]. Isolated CNS PTLD, with negative blood EBV DNA can occur [162].

18.2.10.7 Imaging

Radiological findings show multifocal bilateral lesions in 37–83 %, 77–100 % with ring enhancement. CNS parenchymal lesions are distributed in highest frequency in the cerebral hemispheres (81 %), brain stem (9–15 %), and cerebellum (4–9 %). In the cerebral hemispheres, lesions are most common in the cortex and white matter (33 % each), basal ganglia (13–40 %), and corpus callosum (2 %). The majority of lesions were periventricular (72 %) with predisposition to ependymal location [149, 151, 155, 156]. Isolated leptomeningeal and ependymal enhancement occurred in 10 % patients [152]. MRI can be normal at early stages of the disease [153].

18.2.10.8 Treatment and Outcome

The prognosis is poor. In one summary, survival at 3 years in adults and children was 9 % and 13 %, respectively, vs. 49 % and 56 % in those without CNS involvement ($p < 0.01$). In children, better survival was noted for isolated CNS disease compared with multiple-site involvement (29 % vs. 0 %); however, in adults, the difference was less pronounced (12 % vs. 6 %) [148]. Mortality was 58 % in allo-HSCT patients with CNS PTLD, 40 % died because of CNS PTLD [153]. Overall survival in another study in kidney transplant recipients was 40 %, all the patients had isolated CNS involvement. Surviving patients received multimodal therapy, including immunosuppression reduction, chemotherapy (systemic and intrathecal methotrexate, steroids, and other agents), radiation, anti-B cell antibodies and cytotoxic lymphocyte (CTL) infusion [155]. In another series of 198 SOT patients, 39 patients (20 %) had complete remission, although 17 died later of other causes, and 12 (6 %) were alive and still undergoing therapy [150]. Most of those who had remission got radiation therapy, which appeared to be associated with better outcome and longer survival in several other studies [148, 155]. 41 % of SOT patients were alive at the last follow-up of median 23 months in another study, age was the only predictor of survival [152].

The overall response rate among 84 SOT patients with CNS PTLD was 50/84 (60 %). Complete response was observed in 32/84 (38 %) and partial response in 18/84 (21 %), while 27/84 patients (32 %) had progressive disease with first-line therapy. The most important prognostic factor was response to initial therapy [151].

Reduction of immunosuppression, anti-CD20 antibodies (rituximab), donor lymphocyte infusion or adoptive immunotherapy with EBV-CTL are recommended as first-line therapy for PTLD. Chemotherapy is recommended as a second-line therapy and antiviral agents and IGIV are not recommended [160]. Radiation therapy is an important modality in CNS disease. In one report, outcome was significantly better in patients who received radiotherapy as compared to those who had not: 1 and 5 years survival rates were 71 % and 37 %, compared to 41 % and 28 %, respectively, for the control

group ($p<0.05$) [163]. Rituximab is usually recommended as a first-line therapy in PTLD, but it may be insufficient in CNS PTLD because its low penetration to CSF. After intravenous administration in patients with CNS lymphoma, rituximab is reproducibly detected in the CSF at concentrations that are at most 0.1 % that of matched serum. It is probable that the blood–brain barrier limits the potential efficacy of intravenous rituximab in the prophylaxis or treatment of CNS lymphoma or lymphomatous meningitis [164]. There is some favorable experience with intrathecal (IT) rituximub treatment in resistant cases [165–167]. Seven of eight HSCT patients recovered from CNS PTLD after IT rituximab therapy, five of them achieved complete remission [157]. Most clinical signs and symptoms resolved within 7–10 days after the first IT rituximab administration [157].

18.2.11 Other Causes of Brain Abscess

Pyogenic bacteria, such as *Streptococcus epidermidis*, *Staphylococcus aureus*, *Klebsiella pneumonia,* and *Listeria* were involved in fewer than 10 % of brain abscesses in some series in HSCT and SOT [17, 18, 28, 33, 36].

Tuberculosis is a rare cause of CNS lesions in transplant patients. It was reported in few case reports. It should be considered as a part of differential diagnosis in the endemic areas [168, 169].

Neurocysticercosis is a condition that must be included in the differential diagnosis of patients with CNS involvement and cystic lesions on neuroimaging investigations in transplant recipients, especially patients originating from or traveling to endemic areas [170].

18.3 Meningoencephalitis

Meningoencephalitis in transplant patients may be caused by fungi, bacteria, viruses, and parasites (Table 18-1).

18.3.1 *Cryptococcus neoformans*

18.3.1.1 Pathogen

Cryptococcus neoformans is a ubiquitous saprophytic fungus with worldwide distribution. The fungus is found in nature primarily in association with bird droppings [171].

18.3.1.2 Incidence

Cryptococcal disease, frequently involving the CNS, has an incidence of 2.8 % in SOT population [38]. *Cryptococcus neoformans* was the cause of all five CNS fungal infections that occurred in 51 simultaneous pancreas-kidney transplantations [40]. CNS was involved in 48–72 % of *C. neoformans* infection in SOT recipients; 55 % had *C. neoformans* infection at the CNS site only [38, 172]. The majority of cryptococcal infections (52–64 %) occurred in kidney transplant patients, followed by liver (23.1 %), lung (11.5 %), and heart (7.7 % of cases) recipients [172, 173] in two series in SOT patients. In another report, the incidence of cryptococcal meningitis was higher in heart (2.1–3.5 %) and small bowel (1.7 %) compared to lung, liver, and kidney (0.2–1.1 %) transplant recipients [41, 171]. It is rarely reported after HSCT [174].

18.3.1.3 Time After Transplantation

Cryptococcal CNS infections in SOT recipients occurred in 40 % in the first-year posttransplant. The time to onset varied significantly for different types of organ transplant recipients. The median time to onset after transplantation was 35 months for kidney, 25 months for heart, 8.8 months for liver, and 3 months for lung transplant recipients ($p=0.001$). Overall, cryptococcosis developed in 100 % of the lung, 75 % of the liver, 33 % of the heart, and 30 % of the kidney transplant recipients within 12 months of transplantation ($p=0.002$) [38]. The median time from transplantation to disease was 17.8 months (range 1 month to 15 years) in SOT patients with *Cryptococcus gattii* infection [173].

18.3.1.4 Risk Factors

Patients receiving tacrolimus were significantly less likely to have CNS involvement than patients receiving non-tacrolimus-based immunosuppression (78 % vs. 11 %, $p=0.013$). Furthermore, both tacrolimus and cyclosporine were less likely to be associated with CNS involvement and more likely to be associated with cutaneous infection than azathioprine [38].

18.3.1.5 Clinical Manifestations

Presenting features of transplanted patients with CNS cryptococcosis include headache in 46–62 %; confusion or lethargy in 48–64 %; nausea and vomiting in 50 %, fever/chills in 46 %, malaise in 32 %, meningismus in 14 %, visual loss in 7 %, seizures in 4 %, and coma in 1 % [28, 38, 40, 41]. Subacute meningitis is the usual presentation of cryptococcal infection which appears in 98 % of cases; others have space-occupying lesions [38, 41]. The mean length of symptoms before the diagnosis of meningitis was 17 ± 25d (range 2–30) [171]. The CSF may evidence minimal inflammation and a low cryptococcal antigen titer, leading to an underestimation of the severity of the infection [173].

18.3.1.6 Diagnostic Criteria

Detection of cryptococcal polysaccharide capsular antigen in blood or CSF is the best diagnostic tool. Positive cryptococcal antigen is found in 98–100 % in CSF, and in 86–97.5 % in serum. CSF culture is positive in 77–93 % and blood culture in 3.6–39 %. Positive India ink CSF smear was found in 50–80 % of patients [38, 41, 175]. In another report, serum or cerebrospinal fluid cryptococcal antigen assay was positive in 57.4 % of SOT recipients [172]. Patients with CNS disease had higher serum antigen titers and were more likely to be fungemic (35.6 % vs. 4.8 %, $p=0.0002$) than those without CNS disease [171, 175].

18.3.1.7 Imaging

CT scan and MRI may be normal or with nonspecific findings, including meningeal enhancement [41, 171]. In one study, leptomeningeal lesions was described in 8/55, parenchymal lesions in 6/55 and hydrocephalus 2/55 [175]. In another study, hydrocephalus was the most common finding, found in 7/12 (58 %) patients [176]. Space-occupying lesions (contrast enhancing mass lesions) due to *C. neoformans* were present in 3/125 SOT patients [38]. Immune reconstitution syndrome may contribute to the CNS findings that develop after initiation of the antifungal therapy [175]. Neuroimaging in SOT patients with *Cryptococcus gattii* infection, demonstrated leptomeningeal enhancement more frequent (70 % vs. 7 %) and brain mass was less frequent (0 % vs. 64 %, both $p<0.05$) as compared to normal hosts [173].

18.3.1.8 Treatment and Outcome

The following two options are strongly recommended currently for the treatment of cryptococcal meningitis [177–179]: (1) Amphotericin B, 0.7–1 mg/kg/day, plus 5FC, 100 mg/kg/day, for 2 w, then fluconazole, 400 mg/day for minimum 10 weeks; (2) Amphotericin B, 0.7–1 mg/kg/day plus 5FC, 100 mg/kg/day for 6–10 weeks.

Each of these regimens should be followed by 6–12 months of suppressive therapy with a lower dose of fluconazole (200 mg/day). For those patients receiving long-term prednisone therapy, reduction of the prednisone dosage to 10 mg/day (or its equivalent), if possible, may result in improved outcome to antifungal therapy [177].

Close monitoring for evidence of elevated intracranial pressure is crucial. For patients with elevated baseline opening pressure, lumbar drainage should remove enough CSF to reduce the opening pressure by 50 %, and daily lumbar punctures to maintain CSF opening pressure in the normal range is required. Among patients with normal baseline opening pressure (up to 200 mm H2O), a repeat lumbar puncture should be performed 2 weeks after initiation of therapy to exclude elevated pressure and to evaluate culture status [171, 177].

The use of intrathecal or intraventricular amphotericin B, via a reservoir, may be considered in refractory cases where systemic administration of antifungal therapy fails, i.e., persistence of clinical manifestations of meningitis and elevated cryptococcal antigen titers in CSF [41, 171]. In one study, it was done in 3/28 (11 %) of SOT patients [41].

The mortality is 25–72 % and death occurs at a median of 19 days (range, 5–53 days) after diagnosis [38, 41, 173, 175]. Factors associated with poor outcome are abnormal mental status, absence of headache, parenchymal lesion vs. leptomeningeal [175], renal failure on admission (in the series describing predominantly kidney transplant recipients), and liver allograft failure in liver transplant recipients. The outcome is not correlated with the presence of fever, CSF pleocytosis, positive blood cultures, CSF cryptococcal antigen titer, and different immune suppressive protocols [38, 41, 171]. SOT patients receiving a calcineurin-inhibitor agent have better outcome ($p=0.008$), probably due to antifungal activity of this agent seen in vitro [180]. Mortality did not differ in those who received amphotericin B alone (59 %) and who received amphotericin B plus 5FC (44 %) [38, 175].

18.3.2 Viral Encephalitis

Viruses that can cause posttransplant encephalitis include human herpesvirus 6 (HHV-6), JC virus (JCV), herpes simplex, and others. In one recent study, 32/2628 (1.2 %) allo-HSCT patients developed viral encephalitis. Development of viral encephalitis was associated with the use of OKT-3 or alemtuzumab for T-cell depletion ($p<0.001$). Detected viruses included HHV-6 (28 %), EBV (19 %), herpes simplex virus (HSV) (13 %), JCV (9 %), varicella zoster virus (VZV) (6 %), CMV (6 %), and adenovirus (3 %), more than one virus was identified in 16 % of the patients.

The median onset time was 106 days after HSCT, but onset times were shortest in those with HHV-6 encephalitis and longest in those with JCV-associated progressive multifocal leukoencephalopathy (PML). Alteration of consciousness was the most frequent symptom (81 %), followed by fever (59 %), seizures (34 %), psychiatric disorders such as confusion, psychosis or personality changes (28 %), paresis (25 %), and hypo- or dysesthesia (19 %). Neuroimaging detected abnormalities attributed to viral encephalitis in 53 % of these patients (in 36 % diffuse, in 13 % focal, 4 % both), in 37 % it was normal [181]. The probability of a sustained response to treatment was 63 % with a median survival of 94 days after onset, but significant variation was found between different causative viruses [181]. Mortality was significantly higher in HSCT patients who developed encephalitis as compared to those without encephalitis (66 % vs. 42 %, $p=0.011$).

The mortality rates attributed to viral encephalitis were as follows: 33 % for HHV-6, 33 % for EBV, 80 % for more than one detected virus, 0 % for HSV, and 67 % for JCV.

18.3.3 Human Herpesvirus 6 (HHV-6)

18.3.3.1 Pathogen

HHV-6 is an enveloped DNA virus; see Chaps. 28 and 29.

18.3.3.2 Incidence

In prospective studies, HHV-6 DNA PCR in blood was detected in 47–78 % of HSCT recipients, and 3–6.9 % developed CNS symptoms contributable to HHV-6 [182–188]. In another study, HHV-6 DNA was detected in CSF samples from 5/11 (45 %) HSCT patients with CNS symptoms of undefined origin. On the other hand, it was found in only one of 107 (0.9 %) immunocompromised patients without CNS symptoms and in none of the 11 HSCT patients for whom other causes for the CNS diseases were documented ($p=0.001$) [189].

Acute limbic encephalitis, which is may be associated with HHV-6, developed in 9/584 (1.5 %) HSCT patients, in six of them HHV-6 infection was diagnosed [190].

In SOT recipients, 38–55 % of renal, 22–54 % of liver, 36 % of heart, and up to 57 % of heart–lung transplant recipients have been shown to develop active HHV-6 infection [191, 192]. CNS complications of any etiology occurred in 29 % (9 of 31) of the patients with HHV-6 and 12 % (6 of 49) of the patients without HHV-6 infection ($p=0.06$). However, mental status changes of unknown etiology were significantly more likely to occur in patients with HHV-6 infection (29 %, 9 of 31), compared with those without HHV-6 (6 %, 3 of 49, $p=0.008$) [191]. There are additional sporadic reports on CNS dysfunction related to HHV-6 in other SOT recipients [193].

18.3.3.3 Time After the Transplantation

HHV-6 reactivation usually occur 2–9 weeks after HSCT, which corresponded to the time period when most cases of encephalitis occur [181, 183, 189, 194, 195]. Overall, 33 % of the cases occurred within 3 weeks, 50 % within 4 weeks, and 83 % within 12 weeks of HSCT transplantation [194]. Most HHV-6 infections occur between 2 and 4 weeks after SOT [192]. In liver transplant patients, symptoms of encephalitis occurred within 10 days after transplant in one study, and in another—71 % of the HHV-6 infections occurred within 4 weeks of transplantation [191, 196].

18.3.3.4 Risk Factors

GVHD is a risk factor: overall incidence of grade III and more GVHD was 80 % and 10.5 %, respectively, in those with and without HHV-6 encephalitis ($p=0.003$) [197]. Treatment with alemtuzumab and anti-CD3 monoclonal antibody (BC3) for prophylaxis of acute GVHD was associated with higher incidence of encephalitis [183, 197].

Acute GVHD grades II to IV and adult-mismatched donor were significant risk factors for HHV-6 limbic encephalitis in another report [198].

In addition, acyclovir was associated with a decreased risk of encephalitis after adjustment for receipt of BC3 (RR, 0.3; 95 % CI, 0.1–0.8) [183].

A meta-analysis of studies published during 10 years (2002–2012) demonstrated that prevalences of HHV-6 reactivation and HHV-6 encephalitis were significantly higher in patients receiving umbilical cord blood transplantation (UCBT) as the stem cell source than in patients receiving another stem cell source (72.0 % vs. 37.4 %, $p<0.0001$; 8.3 % vs. 0.50 %, $p<0.0001$, respectively). Monitoring for HHV-6 reactivation was recommended for HSCT patients [199]. Several later studies reported that prevalence of HHV-6 encephalitis and HHV-6 associated limbic encephalitis was significantly higher among patients receiving UCBT than in patients with other sources (7.9–15.7 % vs. 0.7–2.8 %) [104, 187, 198, 200] and in patients who underwent two or more HSCTs (11.9 %), compared to those who received only one HSCT (3.6 %, $p=0.018$) [104].

18.3.3.5 Clinical Manifestations

Clinical manifestations include confusion, somnolence, amnesia, headache, speech abnormalities, seizures, dysesthesia, and coma. Focal neurological findings are less common (17 %). Fever was documented in 25 % (4 of 12) of the patients. The electroencephalogram (EEG) showed mild or severe diffuse abnormalities in some cases and in the others it was nonspecific [185, 189, 194, 197].

HHV-6 infection is associated with acute limbic encephalitis. It is a distinct syndrome which clinical features include acute onset over 1–3 days with marked anterograde amnesia, patchy retrograde amnesia for up to 4 years, seizures, SIADH, and mild CSF pleocytosis. Focal abnormalities in EEG are seen over the temporal or frontotemporal leads in the majority of these patients [190].

Patients with HHV-6 reactivation after HSCT were more likely to develop delirium (adjusted odds ratio = 2.5) and demonstrate neurocognitive decline (adjusted odds ratio = 2.6) in the first 84 days after HCT [201].

18.3.3.6 Diagnostic Criteria

Diagnosing HHV-6 in HSCT patients may be challenging, since neurological complications after transplantation are frequent on the one hand and asymptomatic elevation of CSF HHV-6-PCR may occur [182–184]. Establishing a diagnosis of HHV-6 encephalitis therefore requires the triad of neurological manifestations, evidence of HHV-6 infection in CSF and absence of other pathogens or pathology [197].

18.3.3.7 Laboratory Examination

Examination of CSF may be normal [185], but usually the protein is elevated and sometimes CSF lymphocytosis is found. CSF PCR was positive in all patients at a median of 4700 copies/mL (range, 600–225,000 copies/mL) while HHV-6 PCR in plasma or PBL may be negative [184, 189, 194, 195, 197].

Higher thresholds of HHV-6 DNA were statistically significant predictors of subsequent CNS dysfunction [182]. The quantity of HHV-6 viremia over time was higher in those who developed encephalitis than in those who did not. Patients with levels of HHV-6 DNA 15,000 copies/mL appeared more likely to develop encephalitis than did those with lower levels: 7/29 (24 %) vs. 11/94 (12 %) [183]. Prospective, multicenter study of 230 allogeneic HCT recipients who had twice weekly plasma HHV-6 DNA load demonstrated that none of the 144 patients without high-level HHV-6 reactivation and 7 of 86 patients (8.1 %) with high-level HHV-6 reactivation (plasma HHV-6 DNA $\geq 10^4$ copies/mL) developed HHV-6 encephalitis ($p=0.0009$). For identifying HHV-6 encephalitis, plasma HHV-6 DNA $\geq 10^4$ copies/mL offered 100 % sensitivity and 64.6 % specificity, and plasma HHV-6 DNA $\geq 10^5$ copies/mL offered 57.1 % sensitivity and 90.6 % specificity [187]. In another study, elevated plasma IL-6 concentrations preceding HHV-6 infection in patients who developed CNS dysfunction.[202]

18.3.3.8 Imaging

CNS imaging shows abnormalities only in part of the patients. Abnormalities most commonly involving the temporal lobes and especially the medial temporal lobes may be seen in MRI. All HSCT patients with limbic encephalitis showed focal medial temporal abnormalities in MRI [183, 189, 190, 195, 197].

18.3.3.9 Treatment and Outcome

Treatment usually included foscarnet or ganciclovir [184, 185, 197]. In severe cases, the recovery is slow. In one HSCT study, the confusion improved after median of 11 days, followed by a reduction in seizure frequency and severity, and recovery of short-term memory loss was much slower, with frequent fluctuations in severity over the ensuing weeks [197]. Although some patients developed CNS symptoms on acyclovir prophylaxis [189], prophylaxis with acyclovir was associated with a decreased risk of encephalitis [183]. Breakthrough HHV-6 encephalitis was reported during foscarnet prophylaxis in HSCT patients [203].

Summary of 48 HSCT cases showed full recovery in 43 % patients, 25 % had a progressive course and died within 1–4 weeks of diagnosis and others had improvement, and the others remained with neurological sequella or died later due to other medical problems [195]. In another summary of 14 cases (13 HSCT, 1 liver transplant), the overall mortality was 58 %; in about a half of them the death was considered attributable to HHV-6 infection [194]. HHV-6 encephalitis was independently associated with decreased survival in patients with allo HSCT [200]. In another report, death from HHV-6-limbic encephalitis occurred in 50 % of affected patients undergoing UCBT and no recipients of adult-donor cells [198].

18.3.4 JC Virus

18.3.4.1 Pathogen

JCV is a neurotropic papovavirus virus. Primary infection occurs during childhood in 75 % of the population [204–206]. In immunocompromised patients JCV causes PML, a demyelinating infection.

BK virus known to cause urinary tract problems in transplant recipients was rarely documented as a cause of encephalitis [207, 208].

18.3.4.2 Incidence

Although JC DNAemia or viruria can occur after transplantation, reports on JCV encephalitis are not frequent. The overall JCV DNAemia rate was 5 % in one study in SOT patients, nobody developed PML picture [209]. The rate of JC viruria in kidney transplant patients is 3.8–40 % [210]. Cumulative incidence of PML, however, was 0.027 % following 32,757 renal transplantations or 8.8 cases/100,000 person-years at risk [211]. Sixty-nine cases of PML in SOT and HSCT recipients were summarized in 2011, of them 54 from review of literature from the period 1958–2010; 36 % of them following HSCT, 34 % kidney, and 30 % other SOT [212]; PML was reported in a few allo- and autologous HSCT patients [206, 213]. JCV reactivation occurred in a quarter of allo HSCT patients in one study, 2/20 patients with persistent reactivation developed PML [214]. The incidence of PML in the heart and/or lung posttransplantation population was three cases in 2428 total posttransplantation person-years or an incidence rate of 1.24 per 1000 posttransplantation person-years [212].

A few cases were described following liver transplantation [215].

18.3.4.3 Time After Transplantation

The median time of onset of PML following transplantation (SOT and HSCT) was 17 months and ranged from 1 week to 132 months. Overall, 71 % (17/24) of the cases of PML occurred within 24 months of transplantation.

In another review, the median time to development of first symptoms of PML following transplantation was longer in SOT vs. HSCT (27 vs. 11 months, $p=0.0005$, range of <1 to >240) [212]. Renal transplant recipients seemed to develop these lesions later after transplantation than did other trans-

plant recipients, some cases are reported to occur years after transplantation [204]. Median time was 334 (range 107–1340) days in HSCT patients [181].

18.3.4.4 Risk Factors

Factors associated with PML development were BK polyoma infection, blood transfusions, use of antirejection medications, and higher panel reactive antibody levels before transplant. BK polyoma infection developed in 22.2 % of PML patients vs. 1.1 % of other patients ($p=0.004$), probably reflecting higher level of immune suppression in these patients. Treatment with mycophenolate mofetil was suggested to be associated with PML [211]. Treatment with rituximub is suspected to be associated with JC viremia and cases of PML in SOT and HSCT patients [216, 217].

18.3.4.5 Clinical Manifestations

The clinical presentation of PML in transplant patients is subacute in 75 % of the patients and more abrupt in the others. The most common presenting symptoms are mono- or hemiparesis (50 %); some of them developed tetraparesis with bilateral pyramidal signs: cognitive deficits (47 %), apathy and behavioral changes (46 %), confusion (38 %), and pseudo bulbar changes.

The symptoms usually worsen gradually over a course of days to weeks, and new neurological manifestations may appear, usually resulting from a spread of the lesion or from new lesions at remote sites. Visual symptoms, including homonymous hemianopsia, occurr in 23–29 % of the patients. Sensory symptoms, ataxia, discoordination and memory impairment are described in 29 % of the patients. Other manifestations include speech disturbances (25 %), cerebellar symptoms (19 %), dysarthria (19 %), aphasia (15 %), frontal release signs (12.5 %), and seizures (10–21 %) usually occurring as the disease progressed, and most patients have focal motor seizures. Extrapyramidal signs are described in four patients (17 %). Alexia, dyscalculia, Gerstmann's syndrome, incontinence, dizziness, apraxia, and headache were described occasionally [204, 212].

18.3.4.6 Laboratory

The CSF content is normal in all patients, except for possible mild elevation in protein levels [204]. The sensitivity of PCR for detecting JCV DNA in CSF from HIV-infected patients with confirmed cases of PML was 74 %; the specificity was 96 %. The positive predictive value was 89.5 %, and the negative predictive value was 88.5 % [218].

18.3.4.7 Imaging

MRI is the preferred mode of imaging, showing lesions in all patients. CT scans in patients with PML characteristically reveal hypodense, non-enhancing lesions of the cerebral white matter and mass effect is distinctly unusual [204]. Parietal, occipital, and temporal lobes are the most common sites involved. Neuroimaging abnormalities are usually located in the subcortical white matter but some extend into the deeper white matter or the cortical ribbon. In another study, infratentorial lesions were found in two-thirds of patients [205]. MRI findings can be subtle in HSCT patients because of heavy immune suppression [213].

Imaging characteristics of PML are similar to immunosuppression (cyclosporine or tacrolimus)-associated leukoencephalopathy, and this diagnosis should be excluded.

18.3.4.8 Diagnostic Criteria

Consensus standards for diagnosis of PML have been divided into a "histologic-confirmed" diagnosis, requiring histology from brain tissue, a "laboratory-confirmed" diagnosis, requiring positive PCR from a CSF specimen, and the entity of a "suspected/possible" diagnosis, based upon clinical findings in the presence of characteristic radiologic abnormalities [219].

18.3.4.9 Treatment and Outcome

Mortality is high: 64–71 % of HSCT and SOT patients died, 53–84 % of them directly from PML [204, 212]. Survival rate reported in other studies was 29–41 % [205, 206]. Time from PML onset to death ranged from 2 to 7 months [206]. Median survival following symptom onset was 6.4 months in SOT vs. 19.5 months in HSCT ($p=0.068$) [212]. The majority of the survivors suffer from residual neurological damage, which includes hemiparesis, speech difficulties, dementia, partial incontinence, or myoclonic jerks. Improvement of their neurological symptoms, however, is described, with treatment and time [204]. There were no specific clinical manifestations that differentiate these patients from those who died.

In a summary of post-HSCT patients, 9/15 (60 %) of cases which reported an attempt at therapy reported positive clinical response including survival (median 19 months) at time of case report publication. This positive-reported response was associated with a median time from HCT to symptom onset of 13 months vs. 10 months for cases with reported lack of response (median survival from symptom onset 3.5 months) [213].

In all seven survivors described in one series, the immunosuppressive therapy was reduced to the minimum, and five of them received treatment for PML—cytarabine (three patients), IL-2 (one patient), and cidofovir (one patient). Treatment options used in other patients include cytarabine intrathecally, α-interferon, cytosine arabinoside, mefloquine, donor-derived JCV Ag-specific cytotoxic T lymphocytes [204, 206, 213, 220]. Reduction of immune suppression is important [215, 221].

18.3.5 West Nile Virus (WNV)

18.3.5.1 Pathogen

WNV is a mosquito-borne flavivirus that is transmitted primarily among birds; humans serve as incidental hosts [222]. The disease is endemic in Africa, the Middle East, and southwestern Asia. It has recently spread to Europe and North America [223].

18.3.5.2 Incidence

WNV disease is infrequent in transplant recipients. The seroprevalence of WNV IgM was 2/816 (0.25 %) in one seroprevalence study performed in SOT patients after WNV outbreak in 2002 in Canada [224]. Twenty cases of WNV disease following SOT from infected donor were reported, as well as another SOT and HSCT cases who acquired it from blood product transfusion [222, 225, 226].

18.3.5.3 Time After Transplantation

Patients developed clinical signs of WNV infection 5–37 days after transfusion of infected blood product or donation of infected organ [222, 225, 226]. WNF can occur any time after transplantation, after exposure to infected mosquito.

18.3.5.4 Risk Factors

Transplant recipients may acquire WNV by three mechanisms: (1) from exposure to mosquito (after median incubation time of 13.5 days); (2) from a blood product transfusion (within 4 weeks) and (3) from infected organ that is transplanted (within 4 weeks after the receipt of a component from a donor) [222, 225–227]. Outdoor recreational activity is a risk factor for community exposure to WNV infection [224].

In donor-derived WNV, donors usually reside in areas of increased WNV activity, they are usually asymptomatic [226]

18.3.5.5 Clinical Manifestations

Unlike healthy individuals, in whom the majority of infections are asymptomatic, transplant patients often develop a severe disease. Review of the 20 published cases of organ-derived WNV infection found that 70 % of infected recipients developed encephalitis [226]. The initial clinical symptoms include fever, weakness, myalgia, gastrointestinal complaints, and altered mental status. Neurological involvement appears within 0.5–7 days and the signs and symptoms progress rapidly, reaching a maximum within a few hours to 2 days. Three categories of serious neurological manifestations are described: meningitis, encephalitis, and acute flaccid paralysis. Meningoencephalitis develops in the majority of the patients [223, 225, 228]. Transient seizures occur in up to 30 % of cases. In one series on 11 HSCT and SOT recipients with WNV disease, four developed acute flaccid paralysis; all had severe quadriparesis or quadriplegia associated with hypotonia and areflexia with relatively preserved sensation [228]; four suffered from myoclonus, some had parkinsonian features. All patients who had EEGs manifested a diffuse slowing of variable severity consistent with generalized encephalopathy [228].

18.3.5.6 Diagnostic Criteria

Determination of WNV infection after transfusion requires at least one of the following [222]:

1. WNV-associated illness within 4 weeks after the receipt of a component from a donor with viremia and laboratory evidence of recent WNV infection.
2. Positive test for IgM antibody either without a history or with a possible history of illness compatible with WNV infection.

Determination of WNV-associated illness in a recipient required the following:

- New onset of unexplained fever, meningitis, encephalitis, or acute flaccid paralysis (alone or in combination).
- Laboratory criteria for confirmed recent WNV infection were as follows:
  - Isolation of WNV from tissue, blood, or cerebrospinal fluid.
  - Detection of WNV antigen by immunohistochemical staining or of WNV genomic sequences in tissue, blood, or CSF.
  - Detection of WNV IgM antibodies in a cerebrospinal fluid sample obtained during the acute phase of illness by IgM-capture ELISA.
  - Recent seroconversion, with detection of WNV IgM by IgM-capture ELISA.

18.3.5.7 Laboratory

Usually patients manifested an initial CSF pleocytosis. Positive serum and CSF IgM or PCR confirm diagnosis [223, 225, 226, 228].

18.3.5.8 Imaging

Abnormalities in cerebral white matter, thalami, basal ganglia, brain stem, and other areas are usually demonstrated in MRI [228].

18.3.5.9 Treatment and Outcome

The treatment include (1) reducing immunosuppression; (2) nonspecific antiviral therapy (interferon alfa-2b); (3) passive

immunization with anti-WNV antibody-containing IVIG when available; (4) intravenous immunoglobulin; and (5) ribavirin. The mortality rate was 17–30 %. Among survivors, 36–43 % had full recovery, while others had minor or major sequelae [223, 226–230].

18.3.5.10 Prevention

Transplant patients living in areas of high WNV activity should be advised of the risk of WNV and counseled on the use of protective measures as well as on potentially avoiding outdoor activity during dusk and dawn, when mosquitoes are most active. Testing of donors would be most reliable in areas with a high prevalence of WNV [226], but the screening is controversial, because of the rarity of the disease [228, 231].

18.3.6 *Listeria*

18.3.6.1 Pathogen

Listeria monocytogenes is a Gram-positive, facultative anaerobic non-spore-forming motile intracellular bacillus that is most often transmitted to humans via contaminated foods such as milk and cheese, undercooked meat, or uncooked vegetables [232, 233].

18.3.6.2 Incidence

The incidence of listeriosis during 13 years was 0.5 % (6/1315) allogeneic HSCT in one center, 2/6 cases developed meningoencephalitis [234]. In one review of the reported cases of brain abscess due to *Listeria*, 18 % of 39 patients were renal transplant recipients [235]. A report summarizing 820 cases of *Listeria* CNS infection showed that 21 % were in transplant recipients. It was mainly described in renal transplantation, where it was the most common cause of bacterial meningitis [233]. The great majority of the patients with CNS listeriosis (97 % of 820) had meningitis/meningoencephalitis and the rest had abscesses [233]. CNS involvement is reported from 30 % in HSCT and liver transplant recipients up to 70 % in renal transplant recipients with listeriosis [232, 234, 236].

18.3.6.3 Time After the Transplantation

The time of diagnosis of listerial infection ranged from 3 weeks to 6 years following SOT and HSCT [232, 234, 235].

18.3.6.4 Risk Factors

Graft rejection or transplant dysfunction, as well as CMV infection which cause immune dysfunction are supposed to be risk factors for listeriosis [234, 235].

18.3.6.5 Clinical Manifestations

Clinical symptoms include fever (in 92 %), headache, and irritability, altered mental status on presentation, headache, or obtundation [232]. Notably, meningeal signs are found in 70 % [233]. In patients with cranial neuropathies, the IIIrd, VIth, VIIth, XIth, and/or Xth nerves are most commonly affected [233].

18.3.6.6 Diagnostic Criteria

Isolation of the bacterial pathogen from the CSF or from a brain abscess is needed for diagnosis.

18.3.6.7 Laboratory

CSF is characterized by predominance of PMNs and elevated protein; hypoglycorrhachia may be found. Blood cultures are positive in the majority of cases [233, 234].

18.3.6.8 Imaging

Brain imaging can show signs of cerebritis, abscess, and hydrocephalus, but it can be normal [232–234].

18.3.6.9 Treatment and Outcome

A combination therapy of ampicillin with gentamicin, or with TMP-SMX, is recommended. In meningitis, the duration of therapy is 3 weeks, and for brain abscess or rhombencephalitis is at least 6 weeks. The mortality rate is 14–50 %, higher in patients with seizures [232, 233].

18.3.6.10 Prevention

Transplant recipients should be educated about the potential risks associated with consuming unpasteurized milk and soft cheese and should be encouraged to cook meat products thoroughly [233]. The use of TMP-SMX prophylaxis to prevent PCP may decrease the incidence of the infection in the transplant population.

18.3.7 Other Causes of Encephalitis Posttransplantation

18.3.7.1 **Measles inclusion bodies encephalitis** *(***MIBE***)* occurs typically in immunocompromised patients within 1 year of measles infection. Patients usually present with afebrile focal seizures and altered mentation. The seizures tend to be refractory to anticonvulsant therapy, and EEG often shows epilepsia partialis continua. Laboratory studies in MIBE are

nondiagnostic, as CSF parameters are often normal and CSF antibodies to measles are usually undetectable. Imaging studies such as brain MRI and computed tomography are often normal. Diagnosis requires a brain biopsy, confirming the diagnosis of MIBE by RT-PCR for measles virus RNA or by immunohistochemistry. The prognosis is poor, with a 76 % mortality rate, and all survivors manifesting significant neurologic sequelae. Possible treatment options include Ribavirin and Interferon-α [237].

18.3.7.2 **Parvovirus B19** is a rare cause of posttransplant encephalitis or CNS vasculitis as reported in two renal transplant recipients. One of them developed vasculitis with skin eruptions and recurrent episodes of encephalopathy with focal neurological deficits. B19 DNA was detected in blood, bone marrow, and skin biopsy specimens. Repeat cranial MRI during each episode of encephalopathy showed variable focal findings, and MR angiography revealed vasculitic changes with narrowing of the cerebral arteries. Specific IgM antibodies were positive in CSF [238]. The second one developed seizures and signs of encephalitis on MRI, with positive blood parvovirus B19 PCR, probably acquired from the donated organ [239] Intravenous immune globulin and reduction of immune suppression may help.

18.3.7.3 **VZV**. Although reactivation of VZV is a common event in patients undergoing HSCT occurring in 41 % of patients, CNS involvement is rare (2 %), probably because of an efficient antiviral chemoprophylaxis. Cases of meningoencephalitis and large- and small-vessel vasculopathy in HSCT and SOT recipients were described, some of them fatal [240–242].

Median time since transplantation until the onset of symptoms was 234 (range 207–261) days in HSCT patients [181].

Diagnosis is established by positive CSF PCR in patients with neurological manifestations, although interpretation of a positive VZV CSF PCR result is more problematic in patients with concomitant cutaneous zoster involvement, as some studies have reported VZV DNA in CSF of patients with uncomplicated zoster or with neurological disease likely caused by other pathogens [240]. Treatment with acyclovir should be initiated. Acyclovir-resistant VZV in the CSF was reported in HSCT patient, due to thymidine kinase mutation, while the virus remained sensitive in blood [243].

18.3.7.4 **CMV**. Although CMV is a very significant pathogen in transplant patients and CMV disease often involves a variety of organs, CMV CNS disease is uncommon. In one study, definite CMV encephalitis was diagnosed in only 11 of 552 (2 %) autopsies performed in HSCT and SOT recipients [244]. CMV ventriculoencephalitis was reported is four peripheral HSCT patients [245].

Eleven cases of CMV CNS disease, mainly encephalitis, were described. All were late-onset CMV disease, occurring median 210 (range 166–285) days after HSCT transplantation. CT or MRI of the brain commonly showed multiple foci of restricted diffusion or infarction. Three patients had concomitant retinitis; two patients had concomitant CMV disease outside the CNS, either pneumonia or colitis. All had ganciclovir (GCV)-resistant virus. Some developed CMV CNS disease despite clearing of their CMV viremia, thus the absence of viremia does not exclude CMV encephalitis [246]. Only one patient survived. Alternative treatments for CMV include foscarnet and cidofovir. Combination of increased dosage of GCV (7.5 mg/kg twice a day), foscarnet, and CTL was successful in one HSCT patient with CMV encephalitis [247].

18.3.7.5 **HSV-1/2**. Although HSV-1/2 can be responsible for the majority of nonendemic cases of viral encephalitis and is the most common cause of fatal sporadic encephalitis [248], it is rarely reported in transplant patients. In one report, five of 23 herpesvirus-associated CNS diseases in allo-HSCT patients were due to HSV-1. In another report, HSV was responsible for 5/23 (13 %) of all cases of viral encephalitis in allo-HSCT recipients, in two of them HSV was isolated together with other viruses [181]. The onset time of encephalitis was median 66 (range 42–189 days). Three patients in whom HSV was the only virus isolated in CSF survived; two other patients, with multiple viruses, died [181].

Other rare causes of CNS infections include:

1. **Adenovirus** [249, 250]. Meningoencephalitis without viremia was reported in HSCT patients [250].
2. **Rabies** encephalitis. Transmission via transplanted solid organs from infected donor was reported. Incubation period may be prolonged—18 months after transplantation [251, 252].
3. Five clusters of transmission of lymphocytic choriomeningitis virus (LCMV) and an LCMV like arenavirus via SOT have been described. Some cases may progress to meningitis, encephalitis, and other CNS manifestations, with high case fatality rate [253, 254].

18.3.8 Parasite

A few cases of *Acanthamoeba* infection has been reported in patients following HSCT and SOT. It presented mostly as encephalitis and rarely as cerebral abscess [255]. Presentation in both HSCT and SOT was fulminant manner and death occurred within 2 weeks after the onset of neurologic symptoms [256, 257].

Skin lesions (multiple recurrent panniculitis-like subcutaneous nodules) may predate neurologic involvement and provide an opportunity for early diagnosis and treatment [256, 257]. The diagnosis was made by biopsy. There is no effective treatment for *Acanthamoeba*-related CNS infection. The outcome of 90 % of the patients has been fatal, despite various treatment regimens [255]. Survivors may develop disability, such as hearing loss, vision impairment [257]

18.4 Conclusion

CNS abnormalities are frequent after HSCT and SOT. A variety of pathogens may cause CNS infection which is an important and significant component of the differential diagnosis. The clinical manifestations are not specific and cannot distinguish between different infectious etiologies. Laboratory examination may be normal and biopsy is usually not feasible. Imaging can help to divide CNS pathology into two categories: brain abscess and meningoencephalitis, each has certain pathogens causing it. MRI is more informative than CT. Prompt appropriate workup for patients with neurological findings, attempting to identify the infectious cause followed by treatment targeted to the specific pathogen is critical for survival and for minimizing the neurological sequelae.

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19 Gastrointestinal Infections After Solid Organ or Hematopoietic Cell Transplantation

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19.1 Introduction

The gastrointestinal tract is a common site of infection in patients who are immunosuppressed following either solid organ (SOT) or hematopoietic cell transplantation (HCT). Patients undergoing these procedures have many immunosuppressive drugs in common, but hematopoietic cell recipients must also face the toxicity of myeloablative conditioning regimens, absence of cellular immunity pending engraftment, acute and chronic GVHD, and delayed immune reconstitution. Clinical presentations of intestinal infection can be subtle, and diagnosis of specific infections often requires endoscopic biopsy of intestinal mucosa. Noninfectious problems, for example, diverticular bleeding after kidney transplant or intestinal GVHD after allogeneic HCT, may mimic infections and may also coexist with infection.

19.2 Gastrointestinal Infections After Solid Organ Transplantation

The frequency of gastrointestinal complications after SOT is 20–35 %, encompassing graft dysfunction and side effects of immunosuppression including direct side effects, malignancy (often from viral transformation), and infection [1–3] (Tables 19-1 and 19-2). Despite screening measures and antimicrobial prophylaxis, infectious complications remain a major source of morbidity and mortality. Infections occurring in the first month of transplant are distinct from those occurring later. In the first month most infections are those present prior to transplant (e.g., urinary tract infection), those transmitted by the transplanted organ (e.g., CMV infection), and those related to technical complications of the procedure (e.g., ascending cholangitis). After 1 month, opportunistic infections from viruses, fungi, and parasites residing in gastrointestinal reservoirs, immunoprivileged sites, or latent states, along with community-acquired infection, are more likely to occur [4].

19.2.1 Kidney and Kidney–Pancreas Transplant

After renal grafts (KT), gastrointestinal complications, usually infections, are seen in up to 50 % of patients and correlate with long-term survival [47–49]. Intestinal ischemia is more common problem after KT than after other organ transplants, particularly in patients with polycystic kidney disease [50, 51]. A life-threatening infection can lead to discontinuation of immunosuppressive drugs and the renal graft sacrificed, as uremia is treatable by dialysis.

Biliary tract and pancreatic infections (cholecystitis, ascending cholangitis, infected pancreatitis) are common in KT recipients particularly among patients with diabetes [52], related to a high frequency of gallstones and to elevated blood triglycerides, secondary hyperparathyroidism, and medications, (cyclosporine, azathioprine, and prednisone), respectively [53, 54].

Cytomegalovirus (CMV) viremia and gastrointestinal disease are common in KT and KPT, with pancreas recipients at greater risk due to higher levels of immunosuppression [5, 55]. CMV disease is a risk factor for rejection of renal grafts [56]. Preemptive therapy for viremia reduces the frequency of CMV disease [57]; however, after surveillance has ceased, CMV can cause enteritis or pneumonia years after transplant [58]. The peak time for symptoms is about 8 weeks after transplant. In high-risk patients (donor seropositive and recipient seronegative), valganciclovir prophylaxis is now routinely practiced [5, 59]. In high-risk patients receiving prophylaxis kidney retransplant has been identified as a risk factor for developing CMV reactivation [60].

In historical KT recipients there was a 20 % incidence of gastrointestinal hemorrhage likely related to *Helicobacter pylori* infection [61, 62]. With treatment of *H. pylori* before transplant and use of proton pump inhibitors, ulcer formation and hemorrhage are now rare (<5 %) after KT [3].

Five to 10 % of kidney transplant patients require long-term immunosuppressive therapy because of chronic rejection increasing the risk of CMV, EBV, hepatitis viruses, papillomavirus, parasites, and fungi [63, 64]. Severe colitis

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Table 19-1. Microorganism causes of gastrointestinal infection after solid organ transplantation

Organism class	Epidemiology	Clinical presentation/diagnosis	Treatment/prophylaxis
CMV	Most common gastrointestinal infection resulting from reactivation of latent donor or recipient virus [5] Especially common in treatment for organ rejection [4] treatment with mycophenolate mofetil [6] CMV-negative recipients who receive a CMV-positive graft [7–9] and coinfection with immunomodulating viruses, bacteria, or fungi [4, 10] The peak incidence of CMV infection is 4–6 months after transplant once antiviral prophylaxis has been stopped [11]	Gastroenteritis accounts for 80 % of cases of organ-invasive disease [12] The presentation of CMV gastroenteritis varies widely depending on the affected gut segment and can include dysphagia, odynophagia, nausea, vomiting, abdominal pain, GI bleeding, perforation, or diarrhea CMV intestinal infection may also form intraluminal masses, masquerading as a neoplastic lesion [13]	Use of ganciclovir, foscarnet, valganciclovir prophylaxis has significantly reduced the risk [14–18] Prolonged CMV prophylaxis beyond the typical 6 months has also been evaluated in high risk patients to prevent late reactivation of CMV [19] Low risk patients can be placed on surveillance and treated only if positive for CMV DNA, but virologic surveillance can miss CMV gastroenteritis and hepatitis [20]
HSV	HSV1 and HSV2 are the second most common infection resulting from reactivation of latent recipient virus (not donor)	Gastrointestinal manifestation most commonly involves esophageal ulcers presenting as odynophagia, dysphagia, or nausea. Only rarely involves the intestines If left untreated, it can progress to gastrointestinal bleeding and/or perforation Disseminated HSV presenting with fever, leucopenia, and hepatitis can also rarely occur [21]	Prophylaxis with acyclovir, valacyclovir, valganciclovir, or famciclovir has reduced recurrence [22] If antiviral prophylaxis is not used, manifestations of HSV infection can develop in up to 70 % of transplant recipients [21]
EBV	Gastrointestinal manifestations of Epstein–Barr virus (EBV) less common than other herpes viruses	Primary EBV presents more commonly as mononucleosis with fever, pharyngitis, hepatitis, and enlarged lymph nodes in the early postoperative period [23] Secondary EBV presents more commonly in older patients as posttransplant lymphoproliferative disorder, an infiltrative lymphomatous process involving the viscera and central nervous system [24–27]	Reduction of immunosuppression, rituximab, surgery, and/or chemotherapy may be used for lymphoproliferative disorders
HHV-8	Gastrointestinal manifestations of human herpes viruses (HHV-8) less common than other herpes viruses	HHV-8 is oncogenic and can lead to Kaposi's sarcoma, Castleman's disease, and primary effusion lymphomas (a form of non-Hodgkin's lymphoma) involving the gastrointestinal tract and presenting as intestinal bleeding [28]	Reduction of immunosuppression, possibly surgical excision, radiation therapy, or chemotherapy
Enteropathic viruses	Adenovirus, norovirus, rotavirus, and astrovirus until recently have been an underrecognized cause of diarrhea in patients undergoing SOT [29] Shedding can pose an infectious risk to other immunocompromised patients especially in facilities specializing in transplant care [29] Routine testing with PCR for these viruses should be performed in immunosuppressed patients with diarrhea for the purpose of guiding therapy and infection control	Watery non-bloody diarrhea is usually proceeded by nausea, vomiting, and abdominal cramping Can cause both acute and chronic infections and can lead to prolonged symptomatic or asymptomatic viral shedding [29]	A live attenuated vaccine is available for adenovirus types 4 & 7 and rotavirus that can only be administered only prior to transplant [29] There are no vaccines available for norovirus (see Chap. 28) Alcohol disinfectants are not effective for enteric viruses and soap and water should be used Cidofovir or brincidofovir has shown efficacy against adenovirus [29, 30] Nitazoxanide may have activity against norovirus, although no controlled trials have been performed. No antiviral therapies exist for rotavirus or astrovirus

C. difficile	*C. difficile* infection (CDI) represents one of the most common causes of diarrhea in the SOT population and occurs in greater than 10 % of patients [31, 32] Antibiotics and other factors that decrease microbial diversity in the gut microbiota are thought to contribute to the disease by compromising colonization resistance to pathogenic strains of *C. difficile* [33, 34] A hypervirulent strain BI/NAP1/027 has been responsible for many of the outbreaks of CDI and for severe refractory CDI [35]	Watery non-bloody diarrhea often with elevated white count Pseudomembranous colitis on colonoscopy or flexible sigmoidoscopy Diagnosed most often by a positive PCR for DNA coding for *C. difficile* toxins A or B	Treatment usually consists of metronidazole, oral vancomycin, or fidaxomycin [36] Recurrent CDI can be treated with pulsed dose antibiotics [37] Fecal microbiota transplantation has been given in the immunosuppressed population, and in small cases series does not have increased incidence of infectious complications [38] First FMT has cure rates of 78 % in the immunocompromised population and approaches cure rates of 90 % after a second course of FMT [38, 39] Other therapies including investigational antibiotics, monoclonal antibody therapy, vaccine, and microbe-based approaches to treatment are also currently in clinical trials [40] Colonization with nonvirulent strains by administration of spores has been shown to be protective against recurrent CDI [41]
Fungi	Gastrointestinal infections with fungi are most commonly due to *Candida* spp. (*C. albicans*, *C. albicans*, *C. tropicalis*, and *C. krusei*) [42] and less commonly to *Aspergillus* spp Candidal yeast forms can traverse intact small intestinal mucosa into the portal circulation. Mortality from visceral fungal infection is high especially in patients located in the ICU	Gastrointestinal *Candida* infection can manifest as candidal esophagitis presenting most often with dysphagia or odynophagia, sometimes without oral thrush, thus, obviating the need for upper endoscopy for diagnosis Esophageal candidiasis can often present simultaneously with CMV or HSV especially in patients on high levels of immunosuppression In addition, intestinal reservoirs and swallowed yeast forms of *Candida* and aspergillus can translocate the gut leading to peritonitis, fungemia, intra-abdominal abscesses, and invasive mycoses of the liver or pancreas	Antifungal prophylaxis with fluconazole in SOT patients has not been universal due to side effects, emerging resistance, and lack of controlled trials [43] Instead prophylaxis depends on identifying patients that are at high risk for developing infections [44] Fluconazole is more routinely given in liver, lung, pancreas, and intestinal transplants due to the higher incidence of fungal infections [44] Treatment of Candidal infections depends on the specific species, susceptibilities, and nature of infection [44]
Microsporidia	Microsporidia infection is well appreciated in patients with HIV infection, but may be under diagnosed due to low suspicion and lack of detection with routine stool examination in the post-SOT population [45]	Chronic unexplained diarrhea, fatigue, and weight loss Detection of microsporidia requires the use of a modified trichrome stain	Treatment is most often with albendazole
Strongyloides	Pretransplant screening measures of patients that are from or have visited endemic areas (West Indies and Far East) has reduced the prevalence of disseminated strongyloidiasis in the SOT setting	*Strongyloides stercoralis* can lead to fever, abdominal pain, bloody diarrhea, abdominal distension, and nausea Rarely it presents with an acute respiratory illness caused by migration of *S. stercoralis* through the lungs or hyperinfection which is associated with high mortality [46]	Treatment is most often with ivermectin

Table 19-2. Noninfectious causes of gastrointestinal signs and symptoms after solid organ transplantation, by organs transplanted

Kidney and kidney–pancreas	Pancreas	Liver	Heart, lung, and heart–lung	Intestine and intestine–liver
Acute pancreatitis	Acute pancreatitis	Biliary leak	Choledocholithiasis	Anastomotic leak
Biliary leak	Biliary leak	Bowel perforation	Bowel perforation	Graft rejection
Bowel necrosis	Bowel necrosis	Bowel obstruction	Gastroparesis	
Bowel obstruction	Bowel obstruction	Gastrointestinal bleeding	GERD/Dyspepsia	
Choledocholithiasis	Duodenitis	Graft rejection	Pancreatitis	
Duodenitis	Enterocutaneous fistula	Hepatic artery stenosis	Peptic ulcer disease	
Enterocutaneous fistula	Graft vessel thrombosis	Hepatic artery thrombosis	Pseudoobstruction	
Gastrointestinal bleeding	Graft rejection			
Graft rejection	Internal hernias			
Graft vessel thrombosis				
Intestinal ischemia				

and enterocolitis caused by CMV, *C. difficile*, *C. septicum*, cryptosporidia, and enteric bacterial infections have been described in small numbers of KT patients, usually in case reports [65–68].

19.2.2 Pancreas Transplant

Pancreas transplant recipients develop all the infections common to SOT patients, but most of the abdominal problems that develop are related to the surgery and the grafted tissue [69, 70]. *C. difficile* and CMV are most common gastrointestinal infections [71]. Surgical complications are given in Table 19-2 [69] Pancreatitis may develop in the pancreatic portion of the graft, causing nausea and vomiting, bleeding, anastomotic leaks, perforation, and abscesses [69]. Hematuria may be one sign of bleeding of the graft, if bladder drainage is used.

19.2.3 Liver Transplant

Gastrointestinal complications unique to orthotopic liver transplant (OLT) are generally related to the surgery itself (Table 19-2) [72, 73] and recurrence of the underlying liver disease, both infectious (see Chap. 14) and noninfectious.

A higher incidence of bacterial (70 %), viral (20 %), and fungal (8 %) infections are seen in OLT than other solid organ transplants [74, 75], including enteritis, colitis, ascending cholangitis, peritonitis, and intra-abdominal abscess. This high rate of infections may be related to low albumin state (and ineffective opsonization capacity), decreased barrier function of the intestine, disruption of the luminal integrity with transection of the biliary tract, and iron overload [75, 76]. Viral infections are associated with increased bacterial infections [77] and increase the risk of allograft injury and rejection [74, 78].

Most bacterial infections occur within the first 2 months after transplant [75, 79–81]. A range of bacterial prophylactic regiments have met with mixed results in preventing surgical site and deep intra-abdominal infections [75, 82]. Unusual opportunistic infections (Listeria and Mycobacterium) are more prevalent beyond 2 months after transplant [75]. *Clostridium difficile* infects 2.7–15.8 % of OLT recipients leading to high rates of graft loss, total colectomy, and death [75]. OLT patients with hyperbilirubinemia and hypoalbuminemia are at increased risk for bacterial infections (particularly with *Pseudomonas*) in the setting of liver biopsy [83].

CMV infection in OLT recipients is the most problematic viral pathogen, associated with increased morbidity and mortality [84]. Prophylactic use of ganciclovir is associated with an increased incidence of delayed-onset primary CMV disease, associated with increased mortality. Alternatively, surveillance for CMV viremia or DNAemia and preemptive therapy can be effective. Primary CMV infections can occur in OLT patients who were seronegative at the time of transplant [85–87]. A finding of asymptomatic low-level CMV viremia after OLT does not require antiviral therapy, but patients with high-level viremia or CMV disease in the liver, gastrointestinal mucosa, or lungs are treated (see Chap. 25) [88, 89].

Herpes simplex virus, VZV, rotavirus, adenovirus, and norovirus are rarer causes of viral infections in OLT patients. EBV infections and lymphoproliferative syndromes may occur in chronically immunosuppressed OLT recipients. The incidence of adenovirus infection after OLT is 2–6 % and usually involves the transplanted liver, although other organs can be infected in the absence of liver involvement [90, 91]. The incidence of adenovirus infection is lower in isolated OLT than in combined liver and small intestinal transplant [92].

In the past, OLT recipients frequently developed invasive fungal infections with mortality rates ranging from 65 to 90 % [93], related to fungal overgrowth in the gut with translocation. Current rates of fungal infections are less than 10 % [94, 95]. Many risk factors for fungal infection have been identified including dialysis, rejection treatment, CMV infection, biliary tract disease, use of broad-spectrum antibiotics, indwelling Foley catheter, and iron overload [93, 96]. Fungal resistance to fluconazole has led to alternative antifungal regiments (e.g., micafungin and anidulafungin) [97, 98].

19.2.4 Heart, Lung, and Heart/Lung Transplant

Some 50 % of heart (HT), lung (LT), and heart–lung transplantation (HLT) recipients have gastrointestinal complications, with up to 20 % requiring surgery [99–101]. In the first 30 days the most common complications are pancreatitis, gastroduodenal ulcers, pseudoobstruction, and colonic perforation. In the ensuing months diarrhea, gastroesophageal reflux disease (GERD), gastroparesis, dyspepsia, nausea and vomiting, abdominal pain, pancreatitis, CMV, cholelithiasis, ulcers, and hepatobiliary disease are more common [100, 102, 103].

Symptomatic gastroparesis has been described in 25 % of LT recipients and up to 80 % in HLT recipients [104, 105]. The course is often waxing and waning, but in most patients, there is remission of symptoms [104, 106]. Recipients with GERD and/or gastroparesis are at risk for the development of obliterative bronchiolitis [102, 104]. Proton pump inhibitors are useful for reducing reflux; however, if reflux disease is unremitting, laparoscopic fundoplication may be successful [107, 108]. Giant gastric ulcers (>3 cm in diameter) have been described in LT recipients despite use of proton pump inhibitors. Risk factors include bilateral LT, high-dose NSAIDs, high-dose corticosteroids, and cyclosporine. The description of these ulcers suggests decreased mucosal blood flow from stress or CMV endovascular infection rather than NSAIDs as the cause [109, 110].

CMV disease is more frequent after LT and HT than other organ transplants, presenting most often as pneumonia but gastrointestinal CMV infection is also common [9]. LT and HLT recipients have the highest incidence of fungal infection in SOT. Aspergillus is more common than *Candida* species.

Adenovirus infections in LT and HLT patients affect primarily the transplanted organ rather than the gut and carry a high incidence and mortality [111]. HSV, VZV, and EBV cause the same spectrum of problems in cardiac as in other transplantation patients. The most serious complication is EBV-related lymphoproliferative disease. Although most cases are of B-cell origin, T-cell lymphomas have also been described [112].

Patients undergoing LT for cystic fibrosis experience a unique set of gastrointestinal complications [113] including pancreatic insufficiency and secondary biliary cirrhosis which can complicate absorption of immunosuppressive medications such as cyclosporine. Distal intestinal obstruction syndrome occurs in about 20 %. Cystic fibrosis patients may also experience cholecystitis, peptic ulcer disease, GERD, and gallstones. Biliary complications occur more frequently after HT than in the general population. Transplant patients can undergo elective prophylactic cholecystectomy as mortality of biliary disease post transplant is high [114].

19.2.5 Intestine and Intestine: Liver Transplant

Most of the complications of intestinal transplant are related to the underlying intestinal diseases leading to the transplant (usually short bowel syndrome following infarction or extensive Crohn's disease), rejection of the graft, and anastomotic leaks [115]. The level of immunosuppression to prevent rejection of the graft is high, along with the frequency of infection by herpesviruses, bacteria, and fungi. The most common causes of viral enteritis are rotavirus (high-volume watery diarrhea with prolonged viral shedding) [116]; adenovirus (mostly ileal involvement, with severe symptoms) [92, 117, 118]; norovirus (protracted, severe diarrhea) [119]; and CMV (now less common because of ganciclovir prophylaxis but potentially severe) [120]. The presentation of viral infection often overlaps with signs and symptoms of rejection. Hence differentiation between viral infection and rejection is crucial. Two types of malignancy related to immune suppression have been reported, EBV-lymphoproliferative disease and de novo cancers of non-lymphomatous origin [115, 121]. Surveillance for EBV DNA and preemptive treatment reduces the frequency of lymphoproliferative disease. Because the continuity of the intestinal neurons is disrupted by the surgery, intestinal dysmotility and anorexia can be problematic. Gastrointestinal symptoms secondary to de novo food allergy has been reported in three intestinal transplant recipients [122].

19.3 Gastrointestinal Infections Before and After Hematopoietic Cell Transplantation (HCT)

HCT recipients are prone to many of the same gastrointestinal infections as SOT recipients, but noninfectious intestinal complications are much more common after HCT (Table 19-2) [123]. The current approach to gastrointestinal infection after HCT emphasizes pretransplant screening for infection, prophylaxis, early recognition of infection using molecular methods, and preemptive therapy. Compared to past HCT experience, gut infections are now less common and only rarely cause death.

19.3.1 Gastrointestinal Infections Before the Start of Conditioning Therapy

Unlike SOT candidates, HCT candidates are often immunocompromised and have low platelets prior to transplant due to chemotherapy or the underlying disease process, for example, a hematologic malignancy or immune disorder. Pretransplant gastrointestinal problems can be infectious in origin and require evaluation prior to transplant [124].

In addition to the common causes of upper gastrointestinal bleeding (gastroduodenal ulcers related to *H. pylori* or NSAID use, Dieulafoy lesion, erosive esophagitis), upper sources of bleeding can be due to mucosal infection caused by CMV, HSV, VZV, or *Candida* spp. [124]. In addition to the usual causes of colonic bleeding (inflammatory bowel disease, colorectal cancer, telangiectasias, and diverticula),

bleeding can also be due to infection caused by CMV, *Entamoeba histolytica,* Clostridium *septicum* (typhlitis), and rarely *Clostridium difficile.* Ideally intestinal ulcerations should be healed prior to undergoing HCT as bleeding will likely worsen in the setting of more profound thrombocytopenia with conditioning therapy. Interestingly, *H. pylori* infection in one retrospective study inversely correlated with the development and severity of GVHD [125].

Patients with immune deficiency disorders and immunosuppression caused by hematologic malignancy or its treatment may also present with acute onset diarrhea. In addition to the common causes of diarrhea (irritable bowel syndrome, ulcerative colitis, and Crohn's Disease), infectious causes should be given special consideration including *E. histolytica*, *Strongyloides*, *Giardia lamblia*, cryptosporidia, *Clostridium difficile*, CMV, rotavirus, and adenovirus [126–129]. Some infections like Cryptosporidiosis may be resistant to therapy in an immunosuppressed patient [130], but restoration of normal immunity after allogeneic HCT can effect clearance of cryptosporidia [131]. Typhlitis is a syndrome of cecal edema, mucosal friability, and ulceration in neutropenic patients, often associated with polymicrobial sepsis and high mortality if left unrecognized and untreated; its cause is usually intestinal clostridia infection, particularly *C. septicum* [132, 133]. Typhlitis occurs in the setting of induction chemotherapy and is sometimes difficult to distinguish from the direct toxic effects of the chemotherapeutic agents. Typhlitis has become far less frequent since physicians began prescribing empiric antibiotics that cover clostridial organisms in patients with right lower quadrant pain.

Pain near the anal canal in a granulocytopenic patient is due to bacterial infection until proven otherwise. Administration of broad-spectrum antibiotics, including anaerobic coverage, is adequate treatment in most cases, with surgical incision and drainage reserved for progressive infections [134]. Extensive supralevator and intersphincteric abscesses may be present without being apparent on external examination but can be diagnosed by computed axial tomography (CT), magnetic resonance imaging (MRI) or transperineal sonography. Less commonly, perianal inflammation and ulcers may be due to HSV, CMV, and fungal infections [135].

19.3.2 Gastrointestinal Infections During the First Year After HCT

After HCT, gastrointestinal infections are now far less common than gut mucosal and liver injury caused by noninfectious diseases such as the effects of conditioning therapy, medication side effects, and GVHD [136]. This is largely due to prophylactic regimens. We also have a better understanding of some of the factors that predispose patients to GVHD and superimposed infections such as vitamin D deficiency [137]. When infections do occur, they usually develop in the background of these other gut diseases.

High-dose conditioning therapy damages the oropharyngeal and gastrointestinal mucosa, resulting in oral mucositis anorexia, and diarrhea. Oropharyngeal mucositis may extend into the esophagus, causing dysphagia and painful swallowing that mimics infectious esophagitis [138]. Esophageal infections have almost disappeared as problems after transplant because of prophylaxis against HSV, VZV, and *Candida* species and preemptive therapy in patients with CMV DNAemia.

Anorexia and nausea caused by conditioning therapy varies in its intensity (myeloablative regimens causing more severe and more protracted gut mucosal damage) and may persist beyond day 20. These symptoms are also common complications of transplant medications such as calcineurin inhibitors, sirolimus, mycophenolate mofetil, azole antifungal drugs, trimethoprim–sulfamethoxazole, nystatin, and rarely now, amphotericin B. In years past, herpesviruses were common causes of nausea, vomiting, and anorexia [139], but with the exception of sporadic cases of CMV disease, herpesvirus infections of the upper gut have largely disappeared.

Acute GVHD of the intestine and liver is an immunologic disorder that results from donor lymphoid cells reacting against host tissues and usually has its onset 15–40 days after transplant [136, 139–141]. Gut GVHD can occur earlier if prophylactic medications (e.g., methotrexate, cyclosporine, tacrolimus, mycophenolate mofetil) are not given, or later, following reduced-intensity conditioning regimens [142]. In most patients, acute GVHD diminishes by day 100, although in some it can be protracted. Intestinal manifestations of acute GVHD include nausea and vomiting, profuse watery diarrhea with protein loss, abdominal pain, bleeding, and ileus [123]. The diagnosis of acute GVHD can usually be made on clinical grounds and confirmed by biopsy of target organs. The syndrome of chronic GVHD occurs 3–9 months after transplantation. Intestinal problems of chronic GVHD include esophageal desquamation and stricture formation, bacterial overgrowth in the small intestine, and chronic cholestatic liver disease [143]. MMF can cause intestinal inflammation and ulcerations in a presentation that is difficult to distinguish from GVHD [144]. Oral potassium replacement among other medications can result in esophageal ulcers. Antibiotic use can lead to alterations in the intestinal flora and promote intestinal domination with certain fungi and gram-negative organisms [145]. This may predispose patients to antibiotic-associated diarrhea, *C. difficile* colitis, *C. septicum* typhlitis, and bacteremia (see Chap. 52).

The most common organisms causing gastrointestinal infection after HCT are viral (CMV, norovirus, adenovirus, rotavirus, and astrovirus) and bacterial (*C. difficile*) and they commonly develop in patients with GVHD [146]. Gut infections that were prevalent 30 years ago have largely disappeared because of changes in practice—low microbial foods during extreme immune suppression, prophylaxis of common infections, and microbial surveillance with preemptive therapies [147]. Before fluconazole prophylaxis, problems formerly caused by fungal infection (esophagitis, enteritis,

portal fungemia, bleeding ulcers, and hepatobiliary disease) were not uncommon [148, 149].

Before the advent of serological testing for CMV and preemptive treatment with ganciclovir, gastrointestinal CMV was the most problematic of the herpes viruses [150]. While valganciclovir prophylaxis showed no advantage over PCR-guided preemptive therapy [151], other next generation therapies with fewer side effects than currently used prophylactic antivirals are in Phase III clinical trials and are likely to be used widely as routine prophylaxis in the near future [30, 152]. CMV disease usually presents as nausea and vomiting between 40 and 60 days after transplantation. CMV may be recovered from ulcerations throughout the intestine even when molecular methods cannot identify CMV in the bloodstream [150]. CMV may also be associated with pancreatitis and infiltration of neural elements in the intestine [153].

Adenovirus usually causes a mild to moderately severe diarrheal illness, but severe disseminated disease, with fulminant hepatitis and necrotizing enteritis, has been reported with some serotypes of the virus [154–158]. Other enteric viruses that cause diarrhea include *astrovirus, norovirus,* and *rotavirus* [129, 141]. EBV-associated posttransplant lymphoproliferative disease (PTLD) has a frequency of about 3 % [159]. It can develop rapidly in HCT patients, infiltrating the stomach, intestine, mesentery, liver, and spleen [160]. Poor treatment response to rituximab is determined by age greater than 30 years, involvement of extralymphoid tissue, GVHD, poor response to immunosuppressive tapers, and unchanged EBV viremia [159].

The risk of parasitic diseases has decreased in the setting of pretransplantation screening and treatment. If patients are discharged to a less-controlled environment, they may acquire parasites such as *Giardia lamblia* and *Cryptosporidium* organisms, particularly from infected children and drinking water [126, 161, 162]. The diagnosis of cryptosporidial infection—often missed with standard microscopy—is best made by PCR of fecal specimens [128].

19.3.3 Gastrointestinal Infections in Long-Term Transplant Survivors

Intestinal and hepatobiliary complications after the first year are far less common than earlier post-transplant; most of these intestinal problems, however, are not related to infection (Table 19-2). Some patients with extensive chronic GVHD have esophageal desquamation, webs, submucosal fibrous rings, bullae, and long, narrow strictures in the upper and mid-esophagus [143, 163, 164]. The most common symptom is dysphagia; some patients present with insidious weight loss, retrosternal pain, and aspiration of gastric contents. Chronic GVHD may cause intractable esophageal disease if not diagnosed and treated promptly. Patients with protracted acute GVHD often have symptoms that wax and wane with intensity of immunosuppressive therapy for up to 15 years after HCT, with each exacerbation similar to the presenting signs of GVHD that occurred earlier after HCT (satiety, poor appetite, nausea, episodic diarrhea, and weight loss) [165, 166]. Sporadic cases of fungal and rarely viral esophagitis may occur in patients with chronic GVHD on immunosuppressive and antibiotic therapy. Esophageal strictures may be sequela of earlier herpes virus infection or mucositis. There are sporadic cases of gut infection with *C. difficile*, CMV, and rarely *G. lamblia*, *Cryptosporidia*, *rotavirus*, and *norovirus*, in long-term survivors [143, 161, 162, 167].

Squamous cell carcinoma of the esophagus has been reported in HCT survivors, usually with concomitant chronic GVHD of the oropharynx [168]. Myasthenia gravis may also complicate chronic GVHD, with dysphagia as its presenting complaint. Intestinal diseases in cell donors have been reported in their recipients, for example, inflammatory bowel disease and celiac sprue [169, 170]. Diarrhea, steatorrhea, and weight loss secondary to pancreatic insufficiency have developed in some HCT survivors [171].

19.4 Intestinal Microbiota in Transplant Patients

The gastrointestinal microbiota plays an important role in the development of infections after both SOT and HCT [172]. Much of our current understanding of the microbiota's role in SOT comes from work in patients who have undergone orthotopic liver transplantation [173, 174]. The microbiota may predispose patients to nonalcoholic steatohepatitis. In cirrhosis, the microbiota produces metabolites including ammonia that contribute to hepatic encephalopathy [175]. Administration of lactobacillus combined with a high fiber diet has been shown to prevent postoperative infections in liver transplantation [176]. A study evaluating the effect of pretransplant rifaximin on the incidence of post-liver transplant infections found no significant difference [82]. In kidney transplants, increased abundance of *Faecalibacterium prausnitzii* has been associated with escalation of tacrolimus levels [177]. The microbiota also influences the immune system's T cell subtypes and likely has direct impacts on transplant outcomes [178].

In HCT the microbiota is impoverished as a result of administration of systemic antibiotics [179, 180], gut inflammation caused by GVHD [179], and possibly other factors. The degree to which the diversity is decreased at the time of engraftment has been shown to predict all cause mortality after allogeneic HCT [181]. An impoverished microbiota may effect mortality by leading to worsened GVHD [179, 182], increased risk of enteric infections [183], and increased risk of bacteremia [145]. Certain probiotic species like *Lactobacillus* spp. have been shown to have an ameliorating effect on the severity of GVHD [182]. Patients with leukemia vs. other forms of hematological malignancy have been

shown to preferentially develop low diversity states with predominance of *Enterococcus,* but the reason is not known [145]. Increased risk of low diversity may also be associated with comorbid autoimmune conditions in which dysbiosis has been shown to be prevalent [184].

19.5 A Problem-Oriented Approach to Diagnosis of Gastrointestinal Infections After Transplant

19.5.1 Heartburn, Odynophagia, and Dysphagia

The organisms responsible for infectious esophagitis are fungi, viruses, and bacteria, but infections caused by multiple types of organisms are common in severely compromised, neutropenic patients [185]. In contrast, less compromised patients with indolent esophageal infections may present with chronic dyspepsia and dysphagia; these patients rarely have deep fungal infections involving the spleen or liver, probably because of adequate neutrophil function. Less well appreciated as symptoms of esophageal infection are nausea, vomiting, and anorexia, which are typical of infection with herpesviruses. With antimicrobial prophylaxis, preemptive therapy, and close monitoring, esophageal infections have become rare and noninfectious causes of esophageal disease relatively more common (Table 19-3).

19.5.1.1 Fungal Esophagitis

C. albicans is the most common infecting fungal organism, but other *Candida* species, other fungi (*Aspergillus, Histoplasma, Cryptococcus, Blastomyces*), and some plant molds may be found in severely immunosuppressed patients [185–187]. *Candida* esophagitis can be asymptomatic when few adherent plaques are present. Diagnosis is by stains of brushed or biopsied lesions at endoscopy; cultures cannot reliably differentiate among normal flora, colonization, and infection [188], but are useful if an unusual pathogen such as an azole-resistant *Candida* species, *Aspergillus* species, dematiaceous fungi, *Mycobacterium tuberculosis*, or bacterial esophagitis is suspected. Rapid viral cultures should be routine when viral esophagitis is in the differential, even when fungal esophagitis is obvious.

19.5.1.2 Viral Esophagitis

HSV, VZV, and CMV cause acute ulcerative esophagitis in the immunosuppressed patient, presenting with excruciating retrosternal pain in some and in others just nausea, anorexia, mild heartburn, or bleeding [185]. Reflux of acid-peptic juice contributes to the persistence of large ulcers. The diagnosis of HSV esophagitis is made by finding rounded 1–3 mm vesicles in the mid- to distal esophagus, the centers of which slough to form discrete, circumscribed ulcers with raised edges. The discrete ulcerations can coalesce into very large ulcers, presenting difficulty in diagnosis when there is near-total denudement of esophageal epithelium. The endoscopist must attempt to identify HSV-containing ulcer margins or islands of squamous mucosa from which to obtain samples. IHC, rapid viral diagnosis, and PCR are essential for diagnosis when routine histology is equivocal [185] especially in patients with gastric stasis, vomiting, or poor salivary flow (common problems after both HCT and heart–lung transplant). VZV causes typical vesicles and necrotizing panesophagitis in severely immunodeficient patients, with diagnosis by immunohistologic staining, rapid viral cultures, and PCR. The esophageal component of VZV infection may be overshadowed by varicella encephalitis, pneumonitis, and fulminant hepatitis. Immunohistochemical staining and PCR are helpful in differentiating VZV infection from HSV. VZV and HSV esophagitis are rare in patients receiving acyclovir

Table 19-3. Noninfectious causes of gastrointestinal signs and symptoms after hematopoietic cell transplantation, in descending order of frequency

Heartburn, odynophagia, dysphagia	Anorexia, nausea, vomiting	Diarrhea	Abdominal pain	Perianal pain	Gastrointestinal bleeding
Acid-peptic reflux	Mucosal injury from conditioning therapy	Mucosal injury from conditioning therapy	Intestinal pseudo-obstruction	Anal fissure	Acute and protracted acute GVHD
Oropharyngeal mucositis from conditioning therapy	Acute and protracted acute GVHD	Acute and protracted acute GVHD	Acute and protracted acute GVHD	Thrombosed external hemorrhoid	Mucosal injury from conditioning therapy
Chronic GVHD	Medications	Medications (see text)	Mucosal injury from conditioning therapy	Levator muscle spasm	Mallory–Weiss tear
Pill esophagitis	Rarely, brain disorders (neurotoxicity, hematomas)	Intestinal lactase, sucrose deficiency	Biliary pain (sludge, stones)		Bleeding from mucosal biopsy site
Peptic strictures			Hemorrhagic cystitis		Gastric antral vascular ectasia
Post-infection strictures			Acute pancreatitis		Bleeding from diverticula
Severe acute GVHD			Liver pain (SOS)		
Intramural hematomas			Intestinal perforation		
			Intestinal infarction		
			Intramural hematomas (intestine, abdominal wall)		

prophylaxis. In contrast, CMV never infects squamous epithelium but rather subepithelial esophageal cells leading to superficial erosions with serpiginous, non-raised borders in the mid- to distal esophagus. As CMV infection progresses, shallow ulcerations may deepen, extend for 10–15 cm, and even become strictured. Multiple biopsies should be obtained from the bases of the esophageal ulcers, as this is where CMV-infected sub-epithelial fibroblasts and endothelial cells reside [189]. Immunohistochemical staining for early, intermediate, and late antigens can confirm the diagnosis of CMV infection when infected cells are neither megaloid nor inclusion-bearing. These histologic and immunohistologic methods, however, are only about half as sensitive as rapid viral culture methods [189]. If a positive PCR result for CMV DNA is not concordant with viral culture, immunohistology, blood assays for CMV DNA, or endoscopic findings, antiviral therapy should be withheld.

19.5.1.3 Bacterial Esophagitis

Oropharyngeal bacteria may cause esophageal necrosis in patients who lack granulocytes following HCT [190]. Esophageal symptoms, fever, and bacteremia are the usual presenting symptoms; tissue Gram stain is needed for diagnosis. Mycobacterial esophagitis usually presents an extension of pulmonary and mediastinal infection caused by *Mycobacterium tuberculosis*; primary esophageal infection has also been described [185, 191].

19.5.1.4 Noninfectious Causes of Esophageal Symptoms

Infections must be differentiated from noninfectious esophageal disorders (Tables 19-2 and 19-3). It may be difficult to discern the dominant cause of esophageal mucosal injury when both infection and another cause of injury are present. Reflux of gastric contents is particularly problematic after lung or heart–lung transplant and in the presence of Roux-en-Y anastomosis in liver transplant patients. Less common causes of esophageal injury include pill esophagitis, intramural hematomas, and graft-vs-host disease after HCT.

19.5.2 Anorexia, Nausea, and Vomiting

Before effective antiviral prophylaxis and preemptive therapy after SOT and HCT, herpesvirus infections of the esophagus, stomach, or intestine commonly caused loss of appetite, nausea, and vomiting in addition to painful swallowing or diarrhea [189, 192, 193]. VZV and CMV infections may involve visceral neurons to produce a pseudo-obstruction picture with distention and vomiting [194]. Gastric ulcers caused by CMV may fail to heal on acid-reducing medications [195]. Nausea and vomiting are common manifestations of community-acquired viral gastroenteritis and intestinal parasitic infection, especially with *G lamblia* and *Cryptosporidium* organisms [161, 162]. In SOT recipients, *H. pylori* infections, particularly those that cause pyloric channel ulcerations, may cause anorexia, nausea, and poor oral intake, without ulcer pain (syndrome pylorique) [2]. Central nervous system infections such as aspergillus are another cause of nausea and vomiting after HCT; other neurologic signs and, symptoms usually dominate the clinical picture. Diagnosis of gastrointestinal infection as a cause of anorexia, nausea, and vomiting is a three step process: (1) Analysis of stool specimens if diarrhea or abdominal pain is part of the clinical presentation; (2) upper endoscopy for diagnosis of herpesvirus and *Helicobacter pylori* infections; and (3) directed examination of organs that declare themselves to be involved, for example, gallbladder ultrasound when right upper quadrant pain is present, serum lipase and pancreatic amylase when there is epigastric pain, and so on [100, 101, 136]. It is not uncommon to recover CMV from endoscopic biopsies of focal esophageal or gastroduodenal lesions, even when there is no detectable virus in the blood stream.

19.5.2.1 Noninfectious Causes of Upper Gut Symptoms

The differential diagnosis of anorexia, nausea, and vomiting encompasses a long list of noninfectious causes (Tables 19-2 and 19-3). Anorexia and nausea are such protean symptoms that medical judgment must dictate when to aggressively pursue their causes. The more immunosuppressed and the sicker the patient, the more aggressive should be the evaluation. In SOT recipients, anorexia and nausea may be due to organ rejection; gastroparesis (after lung transplant); failure of liver, renal, pulmonary, or cardiac function; visceral inflammation (for example, pancreatitis, cholecystitis, and cystitis). Acute GVHD may also develop after organ transplantation, usually presenting with fever, skin rash, and gastrointestinal symptoms [196–198]. After HCT, myeloablative conditioning therapy causes nausea, vomiting, and anorexia that lasts for 2–3 weeks [138]. After HCT day +20, the most common cause of upper intestinal symptoms is acute GVHD, which causes gastric mucosal edema and erythema [139, 199]. Lymphocytic gastritis resembling GVHD can also be seen in about 12 % of autologous graft recipients [200]. Less common causes of anorexia and nausea after day +20 include disorders of gastric emptying, medication intolerance, and central nervous system lesions [139, 201–204].

19.5.3 Diarrhea

The differential diagnosis of infectious diarrhea in a transplant patient encompasses the same pathogens as in the normal host, as well as some that are very uncommon under ordinary circumstances. However, in the acute care setting, exposure of patients to environmental pathogens is rare

except for *C. difficile* and thus, infection by common enteric pathogens is rare, particularly when patients are following dietary guidelines for safe foods. The exception to this rule is in countries where patients may arrive for transplant already infected by bacterial, viral, and parasitic organisms or be exposed to them after discharge [68, 126]. Infectious diarrhea is often accompanied by a constellation of symptoms (fever, abdominal pain, nausea, vomiting), particularly in SOT patients [68, 205].

19.5.3.1 Bacterial Causes

C. difficile is the most common bacterial cause of diarrhea in hospitalized transplant patients. Colitis caused by *C. difficile* in granulocytopenic patients may be paradoxically mild and lacking typical pseudomembranes perhaps because colitis is largely due to a granulocyte response to clostridial toxins [141, 206, 207]. A more typical clinical course and endoscopic appearance may be seen later after HCT and in SOT patients; severity of *C. difficile* colitis in SOT patients is similar to that in nontransplant patients [208]. With the emergence of more virulent strains of *C. difficile*, more severe colitis is being seen [209]. Available therapies include metronidazole, vancomycin, and fidaxomycin [40]. A proposed probiotic treatment for *C. difficile* colitis, *Saccharomyces boulardii*, may itself reach the bloodstream in patients with immune defects and should be avoided in transplant patients [210]. In refractory immunosuppressed cases, treatment with fecal transplantation has been as efficacious as in non-immunocompromised individuals without increase adverse outcomes [38, 39]. Strict infection control measures in the inpatient, outpatient, and home settings are essential to prevent the transmission of *C. difficile* [211, 212], Relapse is common in the presence of immunosuppression, especially with glucocorticoids [208]. *Recurrent C.* difficile colitis can be treated with pulsed antibiotics and in some cases fecal transplantation [37].

Cord colitis syndrome linked to infection with *Bradyrhizobium enterica* [213] and *Bacteroides fragilis* [214] presents clinically as non-bloody diarrhea 3–11 months after cord blood transplant, histologically characterized by epithelioid granulomas and responsive to metronidazole or fluoroquinolones [215, 216]. Infections with organisms such as such as *T. whipplei* may also be involved [217]. Cord colitis appears to be absent in some centers consistent with lack of exposure to these Z-specific gut pathogens [218].

Diarrhea (often bloody) is seen with neutropenic enterocolitis (typhlitis) caused by *C. septicum* [132, 219]. Mycobacteria, *Aeromonas* species, and enterohemorrhagic *E. coli* have been described as causes of diarrhea in immunosuppressed patients [220, 221]. Bacterial infections not obviously involving the intestine may also cause diarrhea, for example, *Legionella* pneumonia [222] and toxic shock syndrome associated with *Staphylococcus aureus* infection.

19.5.3.2 Viral Causes

Viral infections can result in both acute and chronic diarrhea in the compromised host. Of the herpesviruses, only CMV and rarely HSV infection [223] lead to enteritis and diarrhea. (1) CMV may cause profuse watery diarrhea with protein loss [224, 225]; (2) or an inflammatory colitis with bleeding and pain but less voluminous diarrhea [65, 226]. CMV enteritis does not always result in diarrhea—anorexia, nausea, vomiting, bleeding, and perforation may be the only symptoms [13, 185, 195, 227]. Later CMV infection, after discharge from the transplant center, remains an issue [228, 229]. Although CMV can be found by PCR or viral culture of stool, CMV enteritis is best diagnosed by IHC and rapid viral culture of biopsy specimens from involved tissue [189]. A positive PCR for CMV DNA may represent viral excretion without CMV disease.

Some serotypes of adenovirus cause rapidly progressive necrotizing enteritis associated with severe pulmonary, liver, or renal infection where prompt diagnosis and treatment is necessary to prevent death [2, 154, 156, 230, 231]. Other adenovirus isolates appear to cause less severe mucosal disease, leading to dilemmas about the optimal treatment strategy, particularly when immune suppressive drugs must be continued and the treatment has toxicity [154, 157, 158, 232]. Detection of adenovirus in stool by PCR may be useful in high risk patients [233].

Acquired enteric adenovirus infection with self limited diarrhea and transient fever has been reported in up to 18 % of children and 8 % of adults undergoing HCT [154]. Severe adenovirus enteritis and colitis may be associated with mucosal erosions, ulcerations or bleeding, and may cause abdominal pain and tenderness. Endoscopic diagnosis may be difficult when ileal disease predominates. Adenovirus may also cause pancreatitis in HCT (associated with abdominal pain) [154, 156, 234–237]. In SOT, the source and predominant site of adenovirus infection is the transplanted organ, particularly in children in the early posttransplant period, likely because of absent antibody immunity [90, 232, 237]. Early treatment of adenovirus disease with cidofovir in HCT may be associated with better outcomes, though criteria for early treatment are not fully established. Current criteria for treatment include multiorgan involvement (i.e., viral isolation, or histological documentation, from two or more sites), viremia with clinical signs of disease, or significant (endoscopic, histological, or clinical) enteritis, pneumonia, hemorrhagic cystitis or nephritis. Use of surveillance plasma adenovirus PCR, as well as stool and urine testing in patients with diarrhea or hematuria may be valuable in early diagnosis and preemptive therapy, especially in pediatric patients and patients undergoing T cell depleted transplants [238]. Most patients with isolated stool or urinary adenovirus recover spontaneously, but close monitoring for progressive disease may be prudent [234, 235]. Criteria for treatment in SOT are poorly defined because adenovirus viremia in SOT

commonly resolves spontaneously or with reduction of immunosuppressive therapy, especially in children [90, 117, 237, 239].

Other enteric viruses (astrovirus, rotavirus, norovirus, coxsackievirus), acquired through epidemic exposure or nosocomial transmission, may cause diarrhea in transplant patients [68, 141, 240]; this category is likely to increase in prominence as panels of molecular diagnostic tests become available. These viruses can be associated with prolonged viral shedding for weeks after cessation of symptoms [235, 236]. Astrovirus, a common cause of endemic as well as nosocomial diarrhea in children, has been reported after both HCT and SOT, with a frequency of <5 % of patients with diarrhea. In one prospective study, the most common viral cause of diarrhea after HCT was Astrovirus, which caused a self-limited form of diarrhea [141]. In HCT patients, symptoms may include nausea and anorexia in addition to diarrhea [141, 241, 242]. Rotavirus, a common cause of diarrhea-predominant viral gastroenteritis in children in winter months, causes diarrhea lasting 3–9 days. Prolonged and profuse watery diarrhea of 2 or more weeks' duration is the main symptom attributable to Rotavirus in transplant patients, where the frequency of infection varies widely from center to center (0–1.5 % in adults, higher in children) [116, 243–245]. Other symptoms reported in HCT patients include vomiting, abdominal pain, anorexia, fever, and abnormal liver tests, but without severe enteritis or mortality [244, 246, 247]. Fecal shedding can continue for three or more months after clinical illness. Nosocomial transmission likely accounts for many infections especially on pediatric units [244, 247]. Norovirus is the major cause of adult epidemic viral gastroenteritis [129]. Clues to diagnosis include epidemic exposure and rapid onset of transient vomiting followed by prolonged watery diarrhea [248, 249]. With the exception of CMV, some viruses for which there are no commercial diagnostic tests, rare cases of mycobacterial infection, and EBV-related lymphoproliferative disease involving the small intestine, almost all of the infectious causes of diarrhea can be discovered by analysis of stool specimens using bacterial and viral cultures, PCR (adenovirus, norovirus, cryptosporidia), ELISA (rotavirus, astrovirus, *G. lamblia*, *C. difficile* antigen), and microscopic examination (parasitic diseases, fungal overgrowth). If the lesion is out of the reach of an endoscope, the diagnosis of EBV-related lymphoproliferative disease can be based on finding EBV DNA in the bloodstream and a mass consistent with lymphoma on imaging.

19.5.3.3 Fungal Causes

In the minimally compromised patient, intestinal fungal overgrowth can be a cause of watery diarrhea [250]. In the absence of antifungal prophylaxis, patients with prolonged granulocytopenia may develop diarrhea caused by mucosally invasive fungal infections [251]. Wide use of azoles in the peritransplant period has eliminated gastrointestinal infections caused by *Candida albicans*, but mucosal infection by molds and other *Candida* species can now be seen [252].

19.5.3.4 Parasitic Causes

Parasitic infections have probably been under diagnosed as a cause of chronic diarrhea in transplant recipients because of insensitive tests [68]. Accurate tests for organisms such as *Giardia lamblia*, *Cryptosporidium*, *Enterocytozoon bieneusi*, *Isospora belli*, and *Strongyloides stercoralis* are now available [128, 130, 253–256]. Cryptosporidial infection may mimic GVHD after HCT [127]. Cryptosporidial infections can be eliminated if the underlying immune deficiency disappears [131]. *Strongyloides stercoralis* enteritis may become exacerbated during immunosuppressive therapy, causing diarrhea and hyperinfection syndrome [238]. *Blastocystis hominis* and *Enteromonas hominis*, long believed to be innocuous commensal parasites, have been blamed for persistent diarrhea in some immunodeficient patients.

19.5.3.5 Noninfectious Causes (Tables 19-2 and 19-3)

Noninfectious causes of diarrhea that are common to all transplant patients include magnesium salts to correct renal magnesium wasting; mucosal toxicity caused by mycophenolate mofetil [144] or brincidofovir, an investigational broad-spectrum antiviral agent [30]; the pro-motility side effects of the macrolides tacrolimus and sirolimus [257, 258]; and antibiotics that depress the colonic flora (removing the ability of these bacteria to salvage carbohydrate and thus, causing osmotic diarrhea after carbohydrate ingestion). The gut toxicity of mycophenolic acid delayed release tablets is considerably less than that of MMF [259]. After HCT, diarrhea is caused by mucosal injury from myeloablative conditioning regimens and from acute GVHD [141].

19.5.4 Abdominal Pain

Pain caused by some intestinal infections and intra-abdominal infection resulting from perforation may be a harbinger of a rapidly fatal illness in immune suppressed patients [47, 136, 260, 261]. Perforation is most common in the gastroduodenal region and the colon but can occur at any site in the intestine. Plain abdominal X-rays and helical CT will determine whether a perforation has occurred but the site of perforation can remain obscure. Causes of perforation include CMV infection, fungal infection, necrosis of transmural tumors, trauma, and diverticula [2, 47, 226, 261, 262]. Diverticular perforation is particularly common in renal transplant patients [263]. CMV and VZV can also involve neural plexi, causing ileus and abdominal distention [153, 194, 264]. Severe abdominal pain is often the first manifestation of disseminated VZV

infection, which may progress to fulminant hepatitis. PCR for VZV DNA in the bloodstream is the most useful diagnostic test for visceral VZV infection in the absence of skin lesions [265]. Early recognition and institution of acyclovir therapy result in improved survival [194].

Other focal infections of the intestinal tract that present with abdominal pain include phlegmonous gastritis, appendicitis, infections caused by clostridia organisms (*C. difficile, C. perfringens, C. septicum*), and *Aspergillus* vasculitis [266]. Most immunosuppressed patients with appendicitis have right lower quadrant pain, but in some the usual presentation is masked by corticosteroids and the lack of granulocytes. Typhlitis is a localized infection of the cecum and right colon, caused by clostridia toxins (usually *C. septicum*) and closely related to granulocytopenia [132]. Typhlitis has been rarely observed after solid organ transplantation, probably because of preserved granulocyte function [267]. Consideration of this diagnosis should prompt empiric use of antibiotics (imipenem, oral vancomycin) that cover both clostridia organisms and colonic flora that are translocating into pericolic tissues, and surgical consultation in the event of progression [268]. Cecal CMV ulcerations, fungal infection, and acute GVHD in HCT recipients may present similarly but do not have the same poor prognosis as clostridial typhlitis [269, 270].

A radiologic diagnosis of pneumatosis intestinalis (gaseous infiltration of the intestinal mucosa, usually the colon) is likely to be made in a patient with pain who undergoes abdominal plain film or computed tomography, and it does not necessarily represent a severe form of enteritis. In some cases, there may be air in the peritoneal cavity, mediastinum, and portal vein in addition to the intestinal mucosa. Pneumatosis intestinalis has been described in organ recipients and HCT patients. Disease associations have been with viral enteritis (particularly CMV) and GVHD. The abdominal examination and clinical course in many patients is surprisingly benign. There are, however, catastrophic processes that present with gas in intra-abdominal tissues (intestinal infarction, bowel obstruction, and clostridia infections) that must be differentiated on clinical, microbiologic, and occasionally surgical grounds from the more benign form of pneumatosis intestinalis [271].

EBV lymphoproliferative disease occurs in both solid-organ and HCT recipients on high-dose immunosuppressive therapy. Lymphoid infiltrates may present as abdominal pain, ileus, and bleeding. PCR for EBV DNA in the bloodstream may allow preemptive reduction of immunosuppressive drugs and use of rituxan to forestall development of tissue invasion with transformed immunoblasts.

19.5.4.1 Noninfectious Causes (Tables 19-2 and 19-3)

Many episodes of abdominal pain after SOT or HCT are not caused by infection but instead by conditions such as intestinal pseudo-obstruction (caused by mu-opioid and anticholinergic drugs), pancreatitis, cystitis, biliary stone disease, and in HCT patients, the toxicity of myeloablative conditioning therapy, acute GVHD, liver pain caused by sinusoidal obstruction syndrome, and intramural hematomas involving the gut or abdomen. The initial approach to diagnosis of the cause of abdominal pain in an immunosuppressed patient must be tempered by the knowledge that intra-abdominal catastrophes may occur without extreme signs and symptoms and that the time from presentation to death can be very short. Paradoxically, there is also a danger of physicians overreacting to severe abdominal pain from a cause that seldom results in morbidity, for example, intestinal pseudo-obstruction related to mu-agonist opioids, an intramural hematoma of the sheath of the rectus abdominus muscle, or narcotic bowel syndrome.

19.5.5 Perianal Pain

Perianal pain in a granulocytopenic patient is assumed to be caused by bacterial infection of perianal tissues until proven otherwise, and thus, this is a more a problem for HCT patients than after SOT. Infections can be a difficult problem to recognize because there may be little in the way of pus but instead only a painful cellulitis. These infections are usually polymicrobial (aerobic and anaerobic bacteria), arising either from anal crypts or from tears in the anal canal [272]. Extensive supralevator and intersphincteric abscesses may also occur without being apparent on external examination. Early antimicrobial treatment has led to a marked decrease in the need for surgery. If an obvious abscess is present, antibiotics, incision, and drainage usually result in relief of pain and resolution of the abscess [273]. If there is evidence of tissue necrosis, a more aggressive surgical approach may be needed to prevent a fatal outcome. Perineal examinations may be limited by severe pain and by a risk of bacteremia if the patient is granulocytopenic. CT, MRI, and rectal endoscopic ultrasound give accurate views of the anatomy involved if there is a true abscess; the predictive value of a negative imaging test for an abscess is high.(302) However, if an imaging test suggests an abscess or clinical examination suggests infection in a perirectal space, an experienced colorectal surgeon should examine the patient under conscious sedation or anesthesia, with an eye toward surgical drainage if a significant abscess is discovered.

HSV causes painful chronic mucocutaneous ulcerations in patients with immunodeficiency syndromes, especially those with T-lymphocyte defects [274]. In the perianal area, the appearance is of multiple superficial ulcers with raised borders. When these ulcers coalesce and become macerated and secondarily infected, it is often difficult to identify them as viral. In contrast to decubiti, HSV perianal ulcers are painful, have scalloped borders, and occur away from pressure points. The diagnosis is best made by rapid viral culture. Acyclovir treatment is effective, but secondary bacterial or fungal infection may delay healing. Recurrence is common unless acyclovir is continued or immunosuppressive therapy decreased.

19.5.5.1 Noninfectious Causes (Tables 19-2 and 19-3)

There are few noninfectious causes of pain in the perianal area in transplant patients aside from anal fissures, a thrombosed external hemorrhoid, and unusual disorders of smooth muscle (levator muscle spasm, proctalgia fugax). Persistent diarrhea may lead to painful maceration of perianal skin and secondary infection by bacteria and fungi.

19.5.6 Gastrointestinal Bleeding

In the era before effective antimicrobial prophylaxis, viral ulcerations were the most common cause of bleeding in both HCT and organ transplant patients, but in the current era, bleeding from viral ulcers has become uncommon [5, 275]. CMV ulcers in the esophagus are usually shallow, but those in the gastroduodenal, small bowel, and colonic mucosa are deeper and capable of eroding into large vessels [65, 84]. CMV may also cause diffuse gastritis or enteritis similar to that seen in inflammatory bowel disease [58, 223, 276, 277] and rarely present as mass lesions [13]. Duodenal or gastric ulcers that appear to be typical peptic lesions may harbor CMV in the ulcer base and may fail to heal on standard ulcer therapy [195, 278]. If ulcers are in the midgut, a radionuclide blood pool scan or capsule endoscopy can localize the bleeding site, allowing angiographic control or surgical resection if the ulcer is truly solitary and continues to bleed. Endoscopic hemostasis of bleeding CMV ulcers can occasionally be achieved, but this must be accompanied by antiviral therapy; CMV ulcers often require 2–3 weeks for mucosal lesions to heal following effective therapy [193].

HSV may present as bleeding from coalescent herpetic esophageal ulcers without symptoms referable to the esophagus [279]. HSV causes gastric and intestinal necrosis only rarely, usually in patients on high-dose immunosuppressive therapy [223]. VZV causes esophagitis similar to that caused by HSV and occasionally gastric ulcers, but not intestinal mucosal necrosis. EBV does not cause direct ulceration, but in transplant patients, it may lead to a lymphoma-like immunoproliferative disease may present with bleeding submucosal nodules as well as diffuse mucosal infiltration with immunoblasts [223, 280, 281]. Some serotypes of adenovirus cause extensive intestinal mucosal necrosis as well as fulminant hepatitis and multiorgan failure in HCT patients; prompt treatment can be effective [154–158].

Esophageal and intestinal fungal infections are now very uncommon causes of serious bleeding in transplant patients [251, 275]. Exceptions are patients with prolonged granulocytopenia in whom deeper penetration of fungal organisms, particularly molds, can erode into large submucosal blood vessels, leading to massive bleeding [252].

Aside from *H. pylori*-associated ulcers in SOT recipients [3], bacterial gut infection as a cause of severe bleeding is rare. Pseudomembranous colitis caused by *C. difficile* may also present as bleeding, especially in patients with a low platelet count [141, 206]. Bloody diarrhea also occurs with typhlitis (*C. septicum* infection), especially if platelet counts are low [282].

19.5.6.1 Noninfectious Causes (Tables 19-2 and 19-3)

Both SOT and HCT recipients may come to their respective transplant procedures with gut lesions that may bleed after transplant. Minor bleeding after HCT usually disappears when platelet counts stabilize [283]. The current frequency of severe bleeding after HCT is less than 2 %, almost all of which is due to noninfectious causes (GVHD, gastric antral vascular ectasia, mucosal biopsy sites) [275, 283, 284]. Bleeding after SOT is more likely to be caused by infection, especially CMV- and *H. pylori*-related ulcers [72]. Noninfectious causes of severe bleeding include diverticular bleeding (particularly after renal transplant), portal hypertension-related lesions after liver transplant [72], and bleeding from anastomoses (choledochojejunostomy after liver transplant, intestinal anastomoses after pancreatic or intestinal transplant), and ischemic colitis.

Severe intestinal bleeding, defined as enough bleeding to lead to hemorrhagic shock or a fall in hematocrit by >10 % or transfusion requirement of 2 units of blood per day, leads to two imperatives—one is to stop the bleeding and the other to make a diagnosis of the lesion that is bleeding—particularly if the cause is an infection that is not being treated. In practice, this means endoscopic evaluation of the upper intestinal tract, or the colon, or both, and blood-pool radionuclide scans, angiographic studies, or capsule endoscopy when endoscopy cannot localize the bleeding lesion.

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Part IV
Bacterial Infections

20 Gram-Positive Bacterial Infections After Haematopoietic Stem Cell or Solid Organ Transplantation

Malgorzata Mikulska and Claudio Viscoli

20.1 Introduction

Bacteria are the most frequent pathogens causing infection after transplant of haematopoietic stem cells, and Gram-positives cocci have been for decades more frequently isolated than Gram-negative rods. Although in this chapter we have chosen to focus mainly on bloodstream infections (BSI) and pneumonia, other types of infection can be observed in these patients, such as gastrointestinal infections, in particular neutropenic typhlitis, urinary tract infections, usually associated with the presence urinary catheters, skin and soft tissue infections (perianal cellulitis, central venous catheter insertion site infections, fasciitis) and meningitis (*Listeria*). In these patients, bacterial infections are usually correlated to neutropenia and graft vs. host disease (GvHD).

Similarly, in solid organ transplant (SOT) recipients, bacterial infections are the most frequent complications as well, including infections related to the surgical procedure and ICU-related infections. Each type of organ transplant is associated with specific infections, such as cholangitis in liver transplant, mediastinitis in heart transplant, tracheal infections and pneumonia in lung transplant and urinary tract infections in kidney transplant recipients. In all these patients there is a large use of central venous catheters (CVC) which play an important role in healthcare associated bacterial infections.

In the HSCT setting, reliable data on the aetiology of bacterial infections come mainly from the results of blood cultures, since bacteraemia is the most frequent bacterial infection. Microbiological documentation is missing in clinically documented infections such as pneumonia or typhlitis. In SOT recipients, bacteraemia is less frequent, and invasive procedures, such as bronchoalveolar lavage with biopsy in case of lung transplant or bile or fluid cultures from biliary stents or abdominal drainages in liver and pancreas transplants are often required to obtain a microbiological documentation of localised infections.

General epidemiology and risk factors for infections after transplant have been described in Section II. This chapter will first report on the epidemiology of infections caused by Gram-positive bacteria, separately for HSCT and SOT. In the HSCT section we will mainly focus on bloodstream infections and pneumonia, in both cases focusing on the most important pathogens, while in the SOT section we have chosen to describe the general situation, and then to focus on specific types of transplants. Less common localisations and unusual pathogens will be dealt with in a single paragraph for both HSCT and SOT. Therapeutic aspects will be discussed by pathogen and antibiotic, and not by type of transplant.

20.2 Epidemiology of Gram-Positive Infections After HSCT

20.2.1 Bloodstream Infections

BSI affect approximately 5–10 % of autologous and 20–30 % of allogeneic HSCT recipients, with significant variations among centres and among patients in the same centre, depending on different transplantation procedures, such as type of conditioning regimen or prophylaxis of GvHD and antibiotic prophylaxis. The incidence of BSI is the highest during the pre-engraftment neutropenic period, mainly in correlation with oral and enteric mucositis and the presence of a CVC. Staphylococci, Enterobacteriaceae and viridans streptococci predominate in this phase. Later on, after engraftment, BSI are more frequent in case of GvHD, hypoglobulineamia or, again, in association with a CVC. Among Gram-positive bacteria, staphylococci and pneumococci are the most typical. The main risk factors associated with BSI due to single Gram-positive bacterial species are reported in Table 20-1. Trends in the epidemiology of BSI after HSCT reflect changes in prophylaxis and treatment regimens (e.g. antibiotic selection during fluoroquinolone prophylaxis), in transplant protocols (impacting on severity of mucositis), and in the incidence of certain pathogens in the general population (e.g. a decline in infections due to *H. influenzae* and *S. pneumoniae* in countries with high vaccination rates).

The predominant aetiology of bacterial BSI during neutropenia, including the pre-engraftment phase of HSCT, has

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Table 20-1. Main risk factors associated with infections due to Gram-positive bacteria in haematopoietic stem cell transplant recipients

Risk factor	Bacterial species
Oral mucositis	Viridans streptococci Coagulase-negative staphylococci
Enteric mucositis	Enterococci Coagulase-negative staphylococci
Use of central venous catheters	Coagulase-negative staphylococci *Staphylococcus aureus* Corynebacteria
Low performance status Comorbidities	Enterococci
Hypogammaglobulinemia Impaired humoral activity Hyposplenism GvHD	Pneumococci
Fluoroquinolone prophylaxis	Staphylococci Enterococci Viridans streptococci
Use of cephalosporins	Enterococci, viridans streptococci (in case of ceftazidime)
Treatment with beta-lactams	Beta-lactam resistant viridans streptococci
Nasal colonisation with MRSA	MRSA
Colonisation with VRE	VRE

GvHD graft-versus-host disease, *MRSA* methicillin-resistant *Staphylococcus aureus*, *VRE* vancomycin-resistant enterococci.

changed several times over the last 5 decades. While in the 1960s and 1970s Gram-negative rods were predominant, a global shift toward Gram-positives was observed in most transplant centres during the next 2 decades [1, 2]. Reasons for an increase in the Gram-positive to Gram-negative ratio included chemotherapy regimens associated with more oral mucositis, universal use of implantable long term CVCs, and selective antibiotic pressure of third-generation cephalosporins or fluoroquinolones, which are more active against Gram-negatives than Gram-positives [1, 3, 4]. However, in the early 2000 the etiological pattern of pathogens causing BSI started to reverse again, with an increase in Gram-negative bacteraemias, both during the early and late post-transplant phases [5–9]. A similar trend was thereafter reported in numerous centres [10–13]. In more recent years, similarly to what reported for other populations, also in HSCT recipients there has been a significant increase in the proportion of infections caused by multidrug resistant (MDR) bacteria, especially ESBL-producing Gram-negative rods, carbapenem-resistant *P. aeruginosa*, or vancomycin resistant enterococci (VRE) [14–16]. Interestingly, an increase in MDR strains has been seen in some [17], but not all countries [18, 19].

In order to investigate the latest epidemiological trends, in 2011, within the 4th European Conference on Infections in Leukaemia (ECIL-4), we performed a literature review on the aetiology of bacterial BSI in haematology and oncology settings. In addition, a questionnaire was sent to participating centres focusing on the current epidemiology, resistant patterns and recommended empirical therapy [19]. The literature review yielded 29 reports from 13 countries published after 2005, with data from autologous and allogeneic HSCT reported in 14 and 19 papers, respectively [19]. The median year of observation was 2001, and the Gram-positive to Gram-negative ratio was 60 % vs. 40 %, with centres reporting ratios ranging from 85 % vs. 15 %, to 26 % vs. 74 %. Coagulase-negative staphylococci and *Enterobacteriaceae*, mostly *Escherichia coli*, were the most frequent pathogens (approximately 25 % of BSI each, although with great variability from study to study: 5–60 and 6–54 %, respectively), followed by *P. aeruginosa* (10 %, range: 0–30 %), *S. aureus* (6 %, 0–20 %), viridans streptococci (5 %, 0–16 %) and enterococci (5 %, 0–38 %) [19].

The ECIL-4 survey questionnaire obtained answers from 33 centres in 18 countries, mostly reporting data from HSCT recipients (autologous in 32 and allogeneic in 30). The median year of observation was 2008, and the results indicated a further decrease in the Gram-positive to Gram-negative ratio (55 % vs. 45 %), again with important differences by-centre (from 85 % vs. 15 % to 30 % vs. 70 %) [19]. In these 33 centres, the *Enterobacteriaceae* were the most frequent pathogens isolated (median 30 %, range 8–56 %), followed by coagulase-negative staphylococci (24 %, 7–51 %), enterococci (8 %, 0–30 %), viridans streptococci (6 %, 0–22 %) and *P. aeruginosa* (5 %, 0–28 %) [19]. Compared to published data, in the ECIl-4 questionnaire the incidence of *P. aeruginosa* was lower, but the incidence of enterococci was higher [19]. Results of literature review and questionnaire are shown in Figure 20-1. One of the most important characteristics of the epidemiology of bacterial infections in transplant patients is the geographical and inter-centre variability in aetiology and resistance patterns. While there are centres where over 70 % of BSI are caused by Gram-negative rods, there are still other hospitals where 90 % of the isolated bacteria are Gram-positive cocci [11, 12, 18–20].

20.2.1.1 Staphylococci

Staphylococci are the most frequent pathogens causing BSI in HSCT recipients. In this setting, coagulase-negative staphylococci cause approximately 25 % of all BSI, while *S. aureus*, a significantly more virulent species, is isolated in only about 5 % of cases [19]. Variations in the incidence of coagulase-negative staphylococci might be in part explained by the fact that not all studies and centres regarded coagulase-negative staphylococci as the true cause of BSI only if isolated in two consecutive blood cultures [21]. Given their low virulence, coagulase-negative staphylococci are associated with very low attributable mortality, although their treatment is frequently complicated by high rates of resistance to methicillin. In fact, in ECIL centres more than half of the isolated coagulase-negative staphylococci were resistant to methicillin, while the rate of methicillin resistance in *S. aureus* was lower [19]. Similarly, in the ECIL literature review, methicillin

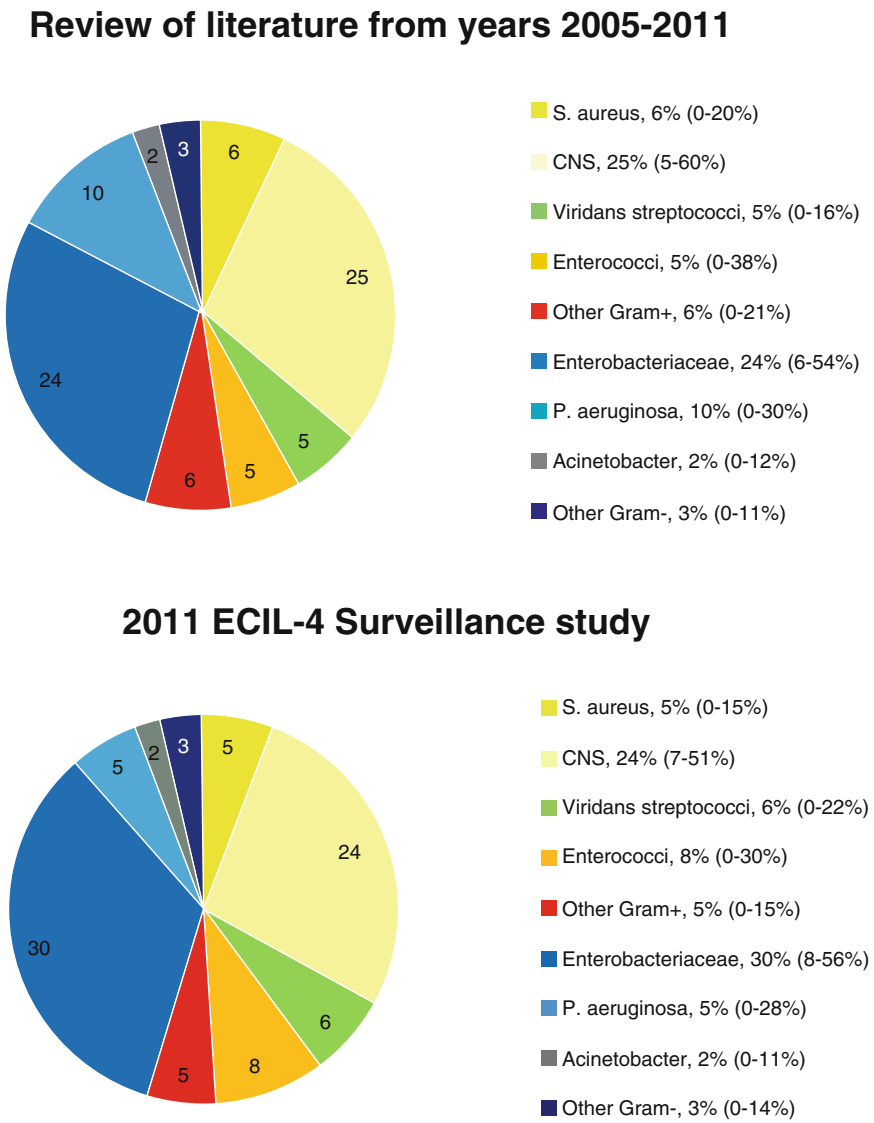

FIGURE 20-1. The aetiology of bloodstream infections according to (**a**) literature review and (**b**) questionnaire survey performed by the European Conference on Infections in Leukaemia reported as median rate (with range) [19]. Reproduced with permission from Mandell, Douglas, and Bennett's Principles and Practice of Infectious Disease 8th ed., by J. E. Bennett, R. Dolin, M. J. Blaser, MD; vol 2; Chapter 309: Prophylaxis and Empirical Therapy of Infection in Cancer Patients, E. Castagnola, M. Mikulska, C. Viscoli, Copyright Elsevier (2014).

resistance was more frequent among coagulase-negative staphylococci than in *S. aureus*, with respective median resistance rates of 80 and 56% [19]. Of note, resistance to methicillin has been reported to be lower in paediatric than adult populations.

Although the overall incidence of methicillin-resistant *S. aureus* (MRSA) bacteraemia is low in HSCT recipients, concerns about high mortality have been raised. In particular, in two MRSA outbreaks, the attributable mortality was very high [22, 23]. In a UK transplant centre, 22 patients became infected and the attributable mortality was 40% in case of early post-transplant infection (2 out of 5 patients) and over 20% in case of late infections [22]. Interestingly, pre-HSCT MRSA colonisation was associated with an increase in morbidity and mortality, even in case of successful mupirocin decolonisation (4 of 11 successfully decolonised patients developed later an MRSA infection) [22]. Hopefully, outside outbreak settings, the outcome of MRSA infections is more favourable, particularly in centres where methicillin-resistant staphylococci are regularly seen and glycopeptides are frequently used in empirical therapy. Infection control measures effective against MRSA and currently recommended by international guidelines include alcohol-based hand hygiene, nasal screening, universal or selective decolonisation, improvement in CVC management and reduction in the use of fluoroquinolones [24, 25]. A reassuring fact about MRSA infections is that, for reasons which remain to be fully understood, since 2004 a steady

worldwide decline in MRSA has been noted in the USA and in several European and Far East countries, despite different infection-control approaches undertaken [26, 27]. Finally, several new therapeutic options against MRSA have been introduced in the last 5 years, including anti-MRSA cephalosporins such as ceftaroline or ceftobiprole, lipoglycopeptides such as telavancin, dalbavancin or oritavancin, or new oxazolidinones, such as tedizolid [28]. Although none of these drugs has been approved for empirical or targeted treatment of infections in neutropenic or transplant patients, they offer a much appreciated alternative for better management of methicillin-resistant infections. Among them, cephalosporins might be particularly attractive due to their historically known efficacy and safety, while some novel lipoglycopeptides might revolutionise outpatient treatment allowing for once weekly administration.

20.2.1.2 Enterococci

Enterococci have emerged as the third most frequent group of bacterial pathogens causing BSI after HSCT, affecting up to 10–12 % of all transplant patients [6, 16, 29–31]. Prophylaxis with fluoroquinolones has been associated with an increased rate of enterococci [32]. However, in our centre, where levofloxacin prophylaxis is routinely used, other independent risk factors for early enterococcal bacteraemia were found, including mismatched related donor or cord blood transplant, low performance score, severe mucositis, pharyngeal enterococcal colonisation and previous empirical therapy with cephalosporins [29]. Interestingly, unrelated donor, cord blood transplant and higher comorbidity scores (together with a diagnosis of acute lymphoblastic leukaemia) were also identified as risk factors for VRE bacteraemia [31]. Compared to other pathogens, enterococcal bacteraemia occurs usually later after transplant. For example the median time to infection from the day of transplant was +4 for viridans streptococci and +11 for enterococci [33]. In many centres, *E. faecium* almost completely replaced *E. faecalis*, with important therapeutic consequences since *E. faecium* is frequently resistant to ampicillin [13, 30, 34]. In some centres, the shift from *E. faecalis* to *E. faecium* has been also accompanied by an important increase in the rate of resistance to vancomycin: in a multicentre Australian study VRE increased from 8 % in 2001–2004 to 64 % in 2007–2010 [30]. The problem of vancomycin resistance is important in HSCT recipients with enterococcal infections, since few therapeutic options are available and high mortality in patients infected with VRE has been reported [35, 36]. In general, the incidence of VRE in Europe is lower than in the USA [6, 13, 19, 26, 33, 34, 37]. In the ECIL-4 survey, 67 % of the interviewed haematological centres reported an incidence of VRE lower than 5 % among all enterococci [19]. On the contrary, in the USA, up to 80 % of *E. faecium* isolates are reported to be VRE and the overall 30-days mortality is very high [16, 31, 35]. It remains controversial whether or not the resistance to vancomycin is the main factor responsible for such a high mortality. In fact, enterococci are poorly virulent pathogens and usually develop in patients with several concomitant clinical problems that are able to affect survival [38]. Therefore, attributing the excess mortality only to the VRE infection in patients with multiple clinical problems might be arbitrary and might simply indicate that VRE are markers of clinical severity [16, 31, 34, 35, 39]. This was clearly suggested by one of the earlier reports, in which all 12 patients with early (average onset day +15) VRE bacteraemia died within less than 3 months from the infection, half of them had blasts at transplant, 80 % had concomitant infections and none achieved platelet engraftment [35]. In larger studies conducted in 76 and 68 patients with VRE bacteraemia, the attributable mortality was, respectively, 6 and 9 % [16, 31]. The view that enterococcal and VRE bacteraemias might be a marker of comorbidities and poor general condition, and as such associated with high overall mortality, is supported by other clinical experiences. In a recent Korean study, for example, a delayed use of adequate antibiotics in case of VRE infection resulted in no difference in 30-day mortality compared to infections caused by vancomycin-susceptible strains in neutropenic patients, and the severity of the underlying disease was the only predictor of poor outcome [40]. In another study in Brazil, the authors found that empirical treatment of neutropenic fever with linezolid had no effect on survival (54 % vs. 42 %) in 100 haematology patients who were colonised with VRE, while predictors of mortality were persistence of neutropenia and GvHD [41]. Finally, in our experience in a cohort of 67 adult allogeneic HSCT recipients with enterococcal BSI, of whom only 13 % had VRE infection, the 30-day mortality for vancomycin-susceptible strains was higher compared to VRE (respectively, 26 and 11 %), whereas the 1-year overall survival was the same in both groups, and significantly lower than in patients with no enterococcal BSI (24 and 65 %, respectively) [42]. These results were compared with an experience of a US transplant centre, where 66 % of patients with enterococcal BSI had VRE. In these patients the 30-day mortality was 38 % for both vancomycin-susceptible and resistant enterococci, while the 1-year overall survival was 48 % for vancomycin-susceptible enterococci, 23 % for VRE and 63 % for patients with no enterococcal BSI [43]. Although enterococcal colonisation (both with VRE and not) has been found significantly associated with enterococcal BSI, its negative predictive value is not very high since only 57 % of VRE-BSI were preceded by VRE colonisation, confirming that this aetiology could not be excluded in non-colonised patients [16, 29]. Screening for rectal carriage of VRE might help to identify patients at highest risk for this infection, yet the positive predictive value for subsequent infection in VRE colonised patients is limited since many of them will not develop a VRE infection. For example in a cohort of HSCT recipients with high prevalence of VRE colonisation (40 %), VRE bacteraemia occurred significantly more frequently in

patients colonised (13/37, 34 %), compared to those with no colonisation, (1/55, 1.8 %, $p < 0.01$), with the positive predictive value of 34 % [44]. As with other MDR bacteria, in order to limit nosocomial spread, patients colonised or infected with VRE should be isolated and contact precautions should be applied.

Treatment of VRE is based on the use of linezolid, for which efficacy data in this setting have been reported. Data on the use of daptomycin have also been reported, although the use of daptomycin in VRE remains investigational [45–48]. Other options are quinopristin–dalfopristin, which is active only against *E. faecium*, and not *E. faecalis* and tigecycline [49]. The new anti-staphylococcal cephalosporins remain inactive against enterococci, while novel glycolipopetides such as telavancin and dalbavancin seem active only against some strains (VanB). For the data supporting the choice between linezolid and daptomycin for VRE infection, see the paragraph on daptomycin in this chapter.

20.2.1.3 Viridans Streptococci

Infections with viridans streptococci have been traditionally associated with oral mucositis in course of chemotherapy (Table 20-1) [especially cytarabine (Ara-C)] and with younger age. Other risk factors were fluoroquinolone prophylaxis, the use of trimethoprim–sulfamethoxazole, antiacid medications and proton-pump inhibitors [50]. A unique aspect of viridans streptococcal bacteraemia during neutropenia consists of the high risk of complicating with septic shock and acute distress respiratory syndrome (ARDS), which usually develops 2–3 days after the onset of bacteraemia [50]. The incidence of these serious complications has been approximately 10 %, varying from 7 to 39 % in different cohorts [50, 51]. Considering that ARDS might be an immunologically mediated phenomenon, early administration of high dose corticosteroids has been studied and reported effective in small single-centre studies in the last 20 years [52, 53]. At present, viridans streptococci are responsible for approximately 5 % of all bacteraemias in transplant recipients. Streptococci are usually susceptible to penicillin and other beta-lactams. However, *Streptococcus mitis*, the species most frequently isolated in neutropenia, is frequently associated with resistance to penicillin and fluoroquinolones [54], making vancomycin the drug of choice for this indication. In fact, in a study which analysed 909 episodes of bacteraemia from 10 randomised clinical trials of antibiotic therapy for infection in patients with cancer and neutropenia in the years 1980–1993, the mortality associated with *S. mitis* bacteraemia was higher if vancomycin was not included in the first line empirical treatment [55]. In that analysis, better survival in patients with Gram-positive bacteraemia who received upfront vancomycin, compared to those in whom vancomycin was added later, was driven exclusively by the benefit observed in 117 cases of viridans streptococcal bacteraemia (mortality 0 % vs. 14 %, respectively, $p < 0.0001$) [55]. Another argument that supported the idea that viridans streptococci might benefit from empirical vancomycin therapy comes from a randomised trial in paediatric patients, in whom vancomycin was added to ticarcillin–clavulanate and amikacin [56]. Higher rates of breakthrough bacteraemia, including 1 case of fatal viridans streptococcal bacteraemia was observed in the non-vancomycin group.

On the other hand, most of the beta-lactams used in empirical therapy today (piperacillin–tazobactam, cefepime or carbapenems) have good activity against viridans streptococci, which is not the case with ceftazidime. In a recent study, only clinical strains with minimum inhibitory concentration (MIC) to penicillin ≥2 μg/mL were found resistant to other beta-lactams [57]. In particular, all isolates with a penicillin MIC=2 were generally susceptible to cefepime, ceftriaxone and piperacillin–tazobactam, while 4 among 17 isolates were resistant to meropenem according to CLSI breakpoints (susceptible according to EUCAST breakpoints). Few strains testing resistant to penicillin according to CLSI (MIC ≥4 μg/mL) were generally non-susceptible to ceftriaxone, cefepime and meropenem, but susceptible to piperacillin–tazobactam. In this study, 17 % of 732 patients were infected with viridans streptococci with a penicillin MIC ≥2 μg/mL, and 98 % of them had at least 1 of the following risk factors: current or past (previous 30 days) use of a beta-lactam as antimicrobial prophylaxis or nosocomial onset of bacteraemia. These might be the subjects who may benefit currently from an empirical treatment with vancomycin. The results of the aforementioned study illustrate well why the newest IDSA guidelines for treating infections in neutropenia do not any longer recommend the use of vancomycin if viridans streptococci are suspected [58]. The association between high penicillin MIC values, clinical outcome and the need for vancomycin treatment have been elegantly discussed in a recent editorial [51].

20.2.1.4 Pneumococci

Pneumococci are an important cause of morbidity and mortality in HSCT patients and the incidence of pneumococcal invasive disease is higher than in the general population, particularly in those with chronic GvHD [59–61]. Nevertheless, the absolute numbers remain low. For example, in a prospective 10-year observational study in Canada, only 14 cases of pneumococcal infection were diagnosed in 1238 adult HSCT recipients (100 % bacteraemia, 71 % with concomitant pneumonia) [61]. This translated into an incidence of 347/100.000 transplanted patients/year, which was higher for allogeneic than autologous HSCT (590 and 199/100.000 transplanted patients/year, respectively), and over 30 times higher than in the general population (11.5 cases/100.000 population/year ($p < 0.00001$). Two HSCT recipients died because of pneumococcal infection, which is similar to the mortality observed in the non-transplant population (14 % vs. 19.5 %). Another recent, more than 10 year-long, retrospective observational

study from Australia identified 23 allogeneic HSCT recipients with invasive pneumococcal disease [62]. The cumulative incidence was 2.3 %, and the incidence density was 956/100,000 transplant/year of follow-up. Of note, *Pneumocystis jirovecii* prophylaxis with trimethoprim–sulfamethoxazole was associated with lower odds of pneumococcal infection. Finally, pneumococcal bacteraemia remains usually limited to the post-engraftment period, accounting for 13 % of all bloodstream infections during this phase, compared to 1 % during pre-engraftment neutropenia [63]. Reasons for an increased risk of pneumococcal infection include long lasting deficit in immunoglobulin production, poor spleen function, and possibly, lack of protection from vaccination. Oral penicillin prophylaxis has been recommended for patients with chronic GvHD or low IgG levels with a grade or recommendation of A III, i.e. a strong recommendation for use, although just based on expert opinions and observational studies [64]. This recommendation might be an extrapolation from the paediatric post-splenectomy literature [65]. However, limitations of long term prophylaxis include suboptimal efficacy, particularly in areas where many pneumococci are penicillin-resistant, poor patient compliance and the risk of developing resistance. Therefore, since the introduction of the conjugated anti-pneumococcal vaccine (PCV), early 4-dose vaccination remains the most widely accepted preventive strategy [64]. The epidemiology of pneumococcal infections has changed after the introduction of conjugated vaccines in the general population, both due to a decrease in the circulation of pneumococci in the community and an increased response to vaccination in HSCT recipients.

20.2.2 Pneumonia

Pneumonia is a frequent infectious complication in HSCT recipients, with an incidence reported in retrospectives studies ranging between 15 and 25 % [66–70]. In a recent prospective observational study, 50 of 169 transplant recipients developed pneumonia. However, in this study, Gram-positive cocci were considered the cause of infection in only five cases, all occurring within 6 months from transplant (2 pneumococci, 2 enterococci and 1 *Nocardia*) [71].

CT scan is the technique of choice for diagnosing pneumonia in the immunocompromised host, Although most CT lesions are not typical for any single bacterial pathogen, cavitary lesions developing during neutropenia are highly suggestive for *S. aureus* pneumonia.. Among rare causes, *Rhodococcus equi* can also be the cause of cavitary and granulomatous lesions in these patients [72].

Numerous acute pulmonary complications may occur in transplant patients, including both infectious and non-infectious causes and it is difficult to obtain an etiological diagnosis. The clinical setting and microbiological analyses, such as cultures of blood samples, sputum and BAL fluid, can be used to provide clues for interpreting abnormal CT findings, although polymicrobial or mixed infections and coexistence of infectious and non-infectious processes (e.g. viral and immunological) further hamper the precise description of epidemiology in this setting [73, 74].

The results of a nationwide prospective study referring to data collected through the Spanish Research Network of Transplant (RESITRA) give insight into these complications [75]. From July 2003 to April 2005, 427 HSCT recipients were followed with a standardised diagnostic protocol for pneumonia. There were 112 episodes of pneumonia and 72 (64.3 %) of them were microbiologically defined. Bacterial pneumonia ($n=32$, 44 %) accounted for more cases than fungal ($n=21$, 29 %) and viral pneumonia ($n=14$, 19 %). The most frequent pathogens isolated in each group were: *E. coli* ($n=7$, 9 %), CMV ($n=12$, 15 %), and *Aspergillus spp* ($n=12$, 15 %). Among bacteria, the most common aetiologies were *E. coli* and *P. aeruginosa*, as previously reported in other studies [66, 76], whereas *S. pneumoniae* was involved in only 5 % of the cases. This low incidence might the result of the routine use of immunisation and prophylaxis. Median time to the diagnosis of pneumonia after transplantation was 66.5 days. The global mortality rate in allogeneic HSCT recipients that had at least one pneumonia episode was 46 % ($n=44$) compared to 13 % ($n=43$) in those without any pneumonia episode ($p<0.01$; relative risk 3.37; 95 % CI: 2.43–4.68). Clinical factors increasing the mortality rate in HSCT recipients developing a pulmonary complication were invasive fungal infection, acute or chronic GvHD, developing pneumonia in the first 100 days after transplantation, acute respiratory failure and septic shock.

In conclusion, bacterial pneumonia is a frequent complication after HSCT procedures, although the aetiological diagnosis is obtained only in a minority of cases. This is due to many causes, including lack of possibility of collecting appropriate respiratory specimens, low yield of blood cultures and an early empirical therapy. Gram-positive bacteria play a significantly less important role than Gram-negatives, with staphylococci and enterococci being most frequently reported.

20.3 Gram-Positive Infections After Solid Organ Transplant

There are several reasons why a solid organ transplant (SOT) recipient can develop infectious complications after the transplant procedure. First of all, the transplant recipient is usually affected by a severe underlying disease, like liver cirrhosis in the liver transplant setting or severe lung and/or heart insufficiency in the heart and lung setting. Other pre-transplant predisposing conditions include diabetes in pancreatic transplant recipients and long-term dialysis before kidney transplants. Given the fact that SOT recipients are

TABLE 20-2. Main risk factors associated with infections due to Gram-positive bacteria in solid organ transplant recipients

Risk factor	Bacterial species
Biliary leakage or intestinal perforation after liver or pancreas transplant	Enterococci
Sternotomy	Staphylococci (mediastinitis)
Length of surgery in any transplant, number of blood units transfused during liver transplant	Staphylococci Enterococci
Surgical technique of liver transplant resulting in intestinal contamination	Enterococci (cholangitis, bacteremia)
Use of central venous catheters	Coagulase-negative staphylococci *Staphylococcus aureus* Corynebacteria
T-cell immunosuppression, lymphopenia after any transplant	*Rhodococcus, Nocardia*
Hypogammaglobulinemia Impaired humoral activity	Pneumococci
Previous treatment with cephalosporins	Enterococci
In kidney transplant, strictures and stents	Enterococci
Colonisation with MRSA	MRSA
Colonisation with VRE	VRE

MRSA methicillin-resistant *Staphylococcus aureus*, *VRE* vancomycin-resistant enterococci.

usually affected by a chronic disease, they are often exposed to hospital pathogens during pre-transplant hospital admissions and might have received numerous courses of antibiotics and immunosuppressive agents, especially corticosteroids. At this point, during transplantation, the patient undergoes a long and usually very invasive surgical procedure, which may last hours and can be complicate by severe bleeding and contamination with intestinal flora (mainly in liver and pancreas transplant). Subsequently, with the incidence depending on the type of transplant, the patient remains in the ICU for a variable number of days, sometimes supported by mechanical ventilation and vasoactive therapy and always receiving artificial nutrition. Urinary catheters and biliary stents might be left in place for several days. Additionally, the patient usually receives an organ from a cadaveric donor and is therefore exposed to the risk of donor-derived infections. Last but not least, immunosuppressive therapy is started immediately after the surgical procedure, with the consequent risk of infections due to opportunistic pathogens and of reactivation of latent endogenous infections. Specific risk factors for infections due to Gram-positive bacteria in SOT recipients are outlined in Table 20-2.

Gram-positive bacteria obviously play an important role in infections in these patients, although an exact evaluation of their impact with respect to other types of pathogens may be difficult. MRSA can colonise the patient before transplant and then cause invasive infections afterwards. Gram-positive cocci are frequent pathogens in surgery- and ICU-related infections, with staphylococci being responsible for healthcare-associated bacteraemia (usually associated with the use of CVC) and pneumonia, and enterococci causing cholangitis and abdominal abscesses, especially in the liver transplant setting [77, 78]. In general, bacteria are the most common pathogens, and the rate of bacterial infections ranges from 5 to 25 % depending on the predisposing factors and type of transplant [79]. The highest incidence of infectious complications is reported for lung and kidney-pancreas transplants, closely followed by liver and heart transplants, while renal transplants are usually associated with a much lower risk [80]. As far as time from transplant is concerned, the risk of nosocomial and opportunistic infections is considered the highest during the first 6 months. In the first month, donor-derived infections as well as those related to the surgical procedure and ICU stay predominate, while in the following 5 months opportunistic infections and infectious reactivations become more frequent, due to the ongoing immunosuppression. Late infections are defined as occurring more than 6 months after SOT and are significantly less frequent than early ones, and are usually caused by the same pathogens that in other patients receiving similar chronic, long-term immunosuppression. However, in a multicentre Spanish study, only slight differences in the aetiology of infectious complications were detected comparing early and late period [80]. Even though late infections were significantly less frequent than early ones, the mortality rate was similar [80].

As already mentioned, Gram-positive infections occur mainly during the early period, accounting for 38 % of pathogens isolated during the first month after transplant, compared to 18 % during the late period [80]. Staphylococcal infections are the most frequent, usually caused by nosocomial methicillin-resistant strains. For example in a recent retrospective study of *S. aureus* bacteraemias in SOT recipients, 70 cases were identified, resulting in an attack rate of 22.9/1000 transplant patients [81]. Early-onset bacteraemia (≤90 days) was more frequent in liver transplant recipients (79 %), when compared with kidney transplants (17 %). All-cause 30-day mortality was 6 %, and was significantly higher in case of pneumonia as the source of bacteraemia (18 % vs. 2 %). Comparing to non-transplant recipients, resistance to methicillin was more frequent (86 % vs. 52 %), and the persistence of positive blood cultures was longer (mean 3.8 vs. 1.6 days); however, quite surprisingly, the 30-day mortality rate was lower [81].

As far as pneumonia is concerned, the Gram-positives are important pathogens, with *S. aureus* being more frequent in the early transplant period, and pneumococci in the late one [61, 82]. Although lower than in case of HSCT, the risk of invasive pneumococcal infections in SOT recipients was nevertheless found to be more than 12-fold higher than in the general population [61]. An interesting study from 90 Turkish SOT recipients who underwent bronchoscopy between 2000 and 2012 reported 46 % of microbiological yield [83]. In these 32 patients, *Mycobacterium tuberculosis*

was the most common pathogen ($n=6$, 7 %), followed by *S. aureus* in 4 (4 %) and *S. pneumoniae* in 2 (2 %) [83].

Enterococci are the second most common Gram-positive bacteria isolated after SOT. In a Swiss cohort, the highest rates of enterococcal infection were found early after liver transplantation (0.24/person-year) [78].

As mentioned, colonisation with resistant Gram-positives is a well-known risk factor for subsequent infection. A recent meta-analysis of 23 studies, mostly (17 %) performed in liver transplant recipients reported that pre-transplant and post-transplant MRSA colonisation significantly increased the risk for MRSA infections with a pooled risk ratio of 5.5 and 10.6, respectively. Similarly, pre-transplant and post-transplant VRE colonisation increased the risk of enterococcal infections almost seven and eightfold, respectively [84]. Thus, preventive strategies should be implemented in order to reduce the risk of resistant infection.

While many infections are common across all SOT receipts, some organ-specific bacterial complications deserve a separate discussion.

In orthotopic *liver transplant* recipients, bacterial, fungál and viral infections are the most frequent complications, affecting up to 68 % of the patients. Bacterial complications present usually early in the post-transplant course, typically occurring within the first 14 days after transplantation [77]. The main reasons for such a high incidence of early infections are prolonged intubation and surgical procedure, with the need to perform several biliary and vascular anastomoses, sometimes positioning intrabiliary devices (Kehr tube, stents). Some of these devices may create a direct communication between the external and the internal (duodenum) environment, with a consequent risk of both endogenous (intestinal flora) and exogenous infections.

For the above mentioned reasons, typical infections after liver transplant involve the biliary tract, and are related to stenosis or bile leakage. Although cholangitis is most frequently caused by Gram-negatives such as Enterobacteriaceae or *P. aeruginosa*, enterococci are not infrequent [85]. In fact, high rates of abdominal infections with any bacteria early after liver transplantation have been reported, but enterococci (in particular ampicillin-resistant *E. faecium*) were the most common pathogens [85, 86].

As far as other Gram-positive infections are concerned, a single-centre experience in 275 liver recipients showed that 7.3 % of them developed *S. aureus* bacteraemia, in median 6 days after transplant [82]. Of note, lungs were the most common primary site of infection, followed by abdomen and biliary tract. Resistance to methicillin was very high (80 %) and a 45 % mortality rate was reported [82]. In another recent experience in 412 adult liver transplants, 71 cases of surgical site infections were diagnosed (17 %) and MRSA was the most frequent single agent isolated, while 25 % of the cases were polymicrobial infections, with high prevalence of enterococci [87]. Half of the surgical site infections were associated with bacteraemia and 23 % with pneumonia; and the reported mortality rate was 14 % [87].

After *lung transplant*, the most important Gram-positive infection is *S. aureus* pneumonia and tracheobronchitis. In fact, Gram-positive infections in this setting are caused almost exclusively by staphylococci. *S. aureus* is particularly frequent and in a recent retrospective single-centre analysis of 596 lung transplant recipients, 18 % ($n=109$) developed *S. aureus* infection [88]. The most common infections were pneumonia (48 %), tracheobronchitis (26 %), bacteraemia (12 %), intrathoracic infections (7 %) and skin and soft tissue infections (7 %). Risk factors included mechanical ventilation for more than 5 days and *S. aureus* colonisation both in the recipient and the donor. The 30- and 90-day mortality rates from the onset of infection were 7 and 12 %, respectively, but there was a significantly higher risk of an acute or chronic rejection in patients with infection [88]. Similarly, in a prospective Spanish multicentre study on pneumonia in 236 lung transplant recipients, its incidence was high, with 72 episodes/100,000 transplants/year, and bacterial aetiology was documented in 83 % of the cases. Pneumonia was caused mainly by Gram-negatives (72 %), with *P. aeruginosa* being the most frequent species (14 cases), followed by *S. aureus* (8 cases); *Nocardia*, isolated in 1 patient, was the only other Gram-positive reported in this cohort [89].

In *heart transplant recipients*, mediastinitis and sternal wound infections are complications unique to this type of transplant. The pathogens are similar to those observed in other patients undergoing cardiothoracic surgery, with the predominance of *S. aureus* and coagulase negative staphylococci [90].

Urinary tract infections are particularly frequent after *kidney transplantation*, as 78 % of all urinary tract infections after SOT develop in renal transplant patients [91]. *E. faecalis* was the second most common isolated pathogen (the first being *E. coli*), and this infection was associated with previous antibiotic therapy or urinary tract instrumentation [92, 93]. *Corynebacterium urealyticum* is an underdiagnosed pathogen in kidney transplant recipients which is associated with encrusting cystitis and pyelitis, with consequent graft dysfunction [94]. When long term incubation and special media providing better diagnostic yield were used in 163 patients, 10 % had *C. urealyticum* bacteriuria, which was related to obstructive uropathy [94]. Similarly to other transplants, wound and CVC-associated infections are frequently caused by staphylococci [92].

Pancreas and small bowel transplant recipients have one of the highest rates of infections after SOT. They are either systemic or localised (intra-abdominal) infections caused by bacteria of enteric origin (for example abscesses in case of enteric drainage of pancreatic secretions or mucosal injury leading to breakdown of bowel-blood barrier with subsequent septic syndromes after small bower transplant). Enterococci are the main Gram-positive pathogens in these settings.

20.4 Other Infections in HSCT and SOT Recipients

20.5 Skin and soft tissue infections in transplant recipients are usually associated either with surgical procedures (particularly in case of SOT) or with the presence of CVC. Gram-positives are the most frequent pathogens, with the predominance of staphylococci. However, the possibility of CVC-associated infection caused by Gram-negatives, in particular *Pseudomonas aeruginosa*, should be always considered in transplant recipients. CVC-related infections include bacteraemia or localised (exit site or tunnel) infections. The exact role of indwelling catheters as a source of fever during neutropenia is difficult to establish since most of blood cultures are drawn only from CVC and not from a peripheral vein. Therefore, the role of CVC should be suspected in case of persistently positive blood cultures, presence of local signs of infection or fever developing precisely after CVC is used. CVC should be promptly removed in case of CVC-associated infection caused by *S. aureus, P. aeruginosa* or fungi. The benefit of CVC removal in case of other CVC-related infections is less clear. In some cases, systemic therapy of CVC-related BSI might be accompanied by local antibiotic intra-CVC therapy, i.e. high concentration of antibiotic solution is left for 8–24 h in each CVC lumen and the catheter is not used (antibiotic lock therapy, ALT). For Gram-positives, clinical experiences with vancomycin, daptomycin and gentamycin have been reported. Prevention and management of CVC-associated infections, including ALT have been recently reviewed elsewhere [95, 96].

20.6 Less Common Gram-Positive Pathogens

Numerous other Gram-positive bacteria may cause infections in immunocompromised patients. In general, in case of low virulence common skin contaminants such as micrococci, corynebacteria or *Rothia* (and similarly to what is done for coagulase negative staphylococci), at least two consecutive positive blood cultures are required in order to consider the case as true bacteraemia, at least in epidemiological studies. In particular, corynebacteria, which are part of skin flora, may occasionally cause bacteraemia, particularly in neutropenic patients with a CVC. Among them, *Corynebacterium jeikeium* is an important species, with a mortality rate in neutropenic patients as high as 34 % (but <5 % in case of bone marrow recovery) [97]. Risk factors for *C. jeikeium* include prolonged neutropenia, the presence of central venous catheter and previous antibiotic therapies [98]. Additionally, *C. jeikeium* is typically resistant to many antibiotics and vancomycin is the most reliable treatment [99]. In the absence of local infection, the removal of CVC does not seem to offer additional benefit to vancomycin therapy [100]. Resistance to new agents such as daptomycin has been occasionally reported [101].

Rothia mucilaginosa, formerly known as *Stomatococcus mucilaginosus or Micrococcus mucilaginosus,* is a Gram-positive coccus which can be easily mistaken for a coagulase-negative staphylococcus on initial culture results. It resides in the oral cavity and upper respiratory tract. It has recently emerged as a cause of life threatening infections in patients with a CVC, with prolonged neutropenia, and mucositis [102, 103]. In addition to disseminated infections and pneumonia, meningitis has been also reported [102, 103].

Bacterial foodborne infections seem infrequent during the first year after HSCT, affecting only 0.3 % of 4069 patients [104]. Listeria was only the fourth most common pathogen, after *Campylobacter, Yersinia* and *Salmonella,* with only two patients developing listeriosis. Also in another cohort, listeriosis was infrequent, with a 13-year incidence of systemic *Listeria* infections of 0.47 % in both HSCT and SOT recipients [105]. Interestingly, only two among six patients had meningoencephalitis, while all had positive blood cultures.

Nocardiosis is a rare bacterial infection occurring in patients with deficient cell-mediated immunity. As such, transplant recipients are at risk, and routine prophylaxis with trimethoprim–sulfamethoxazole prescribed for prevention of pneumocystosis is not effective in preventing nocardiosis. Clinical sites of nocardiosis include lungs, brain or skin, but disseminated infection might also occur. A review on Nocardia infection dedicated exclusively to transplant recipients has been recently published [106].

20.7 Treatment of Gram-Positive Infections in Transplant Recipients

20.7.1 Empirical Therapy for Febrile Neutropenia Targeting Gram-Positive Bacteria

Empirical antibiotic therapy for patients with febrile neutropenia has been one of the main advances in reducing mortality in this setting. The antibiotic regimen must include antibiotics active against Enterobacteriaceae and *P. aeruginosa* since even a short delay in starting treatment might have fatal consequences in these infections [58]. As shown by several clinical trials performed long time ago mainly, but not only, by the IATCG of the EORTC, ceftazidime, cefepime, piperacillin–tazobactam and the carbapenems are all listed as suitable options [58]. A recent trial performed by the Italian GIMEMA group suggested that tigecycline might

be beneficial for better coverage of Gram-negative rods when combined with piperacillin–tazobactam [21].

In the 1970s and 1980s, with an increase in the rate of Gram-positive bacteria causing infections during neutropenia and a rising proportion of methicillin-resistant staphylococci, the inclusion of a glycopeptide antibiotic in the empirical regimen has been proposed with the aim of reducing morbidity and mortality [107–109]. The issue has long been debated. Early data from case-control or historically controlled studies suggested that early initiation of vancomycin in some patients with Gram-positive bacteraemia might have resulted in improved outcome. For example, a survival benefit was reported for patients with viridans streptococcal bacteraemia who received immediately empirical therapy with vancomycin [55]. As a consequence, upfront vancomycin has been recommended in patients with the suspicion of this infection, since severe complications are not infrequent and standard treatment options can be inactive against *S. mitis* [55, 110]. The other side of the coin to consider before recommending upfront glycopeptides because of fear of streptococci is that only a small proportion of febrile and neutropenic patients actually has a viridans streptococcal bacteraemia, and, among them, an even smaller proportion is infected with a penicillin-resistant strain. Cefepime, piperacillin–tazobactam or carbapenems have all satisfactory activity against most viridans streptococci (see the dedicated paragraph).

Strategies for covering resistant Gram-positives include either starting upfront an empirical regimen that contain a glycopeptide or adding a glycopeptide in patients who remain febrile with a standard empirical treatment.

Two studies were performed in order to resolve the doubts about the benefit of including a glycopeptide in the initial empirical therapy. In a large retrospective cohort study, neutropenic patients with Gram-positive infections who did not receive vancomycin before identification of the pathogen did not have worse outcomes [107]. The second study was an IATCG-EORTC prospective randomised clinical trial in 747 patients with fever during neutropenia. The results showed no benefit of adding vancomycin to initial empirical therapy with ceftazidime and amikacin, neither on the length of fever nor on mortality [111].

The second strategy, i.e. adding a glycopeptide in patients not responding to the first line therapy, has been studied in two large placebo-controlled trials. In the first one, a single-centre study, 114 patients were randomised to receive either teicoplanin or placebo after 72–96 h of imipenem monotherapy [112]. The number of patients who became afebrile within 72 h after randomisation was similar in both groups (44.6 % in teicoplanin group vs. 46.6 % in placebo group), but the time to defervescence was not reported. In the second study, an IATCG-EORTC trial, vancomycin (or placebo) was added after 48–60 h of empirical therapy with piperacillin–tazobactam in 165 neutropenic patients with persistent fever of unknown origin or with Gram-positive bacteraemia due to a strain susceptible to piperacillin–tazobactam [113]. Defervescence occurred in 82 of 86 patients (95 %) in the vancomycin group and 73 of 79 (92 %) in the placebo group ($p=0.52$). The difference in the median time to defervescence (the primary endpoint) was not statistically significant [3.5 days (95 % CI 2.7–4.4) in the vancomycin group and 4.3 days (95 % CI 3.5–5.1) in the placebo group ($p=0.75$)]. Gram-positive bacteraemias and the rate of addition of amphotericin B were also similar in both groups. Therefore, these two studies suggested that the empiric addition of a glycopeptide antibiotic is of no benefit in persistently febrile neutropenic patients, in the absence of lung infiltrates, septic shock, or clinically documented infections likely caused by a Gram-positive (such as catheter-related or skin and soft tissue infections).

In fact, when empirical therapy apparently fails and a patient remains febrile, numerous possibilities other than infection with a resistant Gram-positive exist. They include: (1) slow response to initial empirical regimen (time to becoming afebrile might be longer 96 h in patients with a severe infection); (2) infectious but not bacterial cause of fever (usually fungal or viral); or (3) a non-infectious cause of fever such as engraftment syndrome, drug reactions, reaction to infusion of blood products, the underlying malignancy, etc.

The conclusions from the aforementioned studies were confirmed by two large meta-analyses published in 2005 [109, 114]. The first one included 13 studies with 2392 patients and reported no survival benefit for the practice of including vancomycin in the initial regimes, with the disadvantage of more frequent adverse events [109]. The second one, based on data from 14 randomised trials which studied 2413 patients, admitted that slightly higher (odds ratio between 1.6 and 2.2) treatment success without modification of the initial regimen was accomplished if a glycopeptide was included in the empirical therapy [114]. However, no effect on mortality or length of fever was found. Furthermore, adding a glycopeptide to the antimicrobial regimen in the case of persistence of fever for 72 or more hours after the start of the empirical treatment resulted in no increase in treatment success (odds ratio 1.02, 95 % CI 0.68–1.52) [114]. In consideration of the higher rate of adverse effects (odds ratio almost 5), including nephrotoxicity, also this meta-analysis concluded that glycopeptides should not be routinely used as part of the initial empirical treatment of febrile neutropenic patients [114].

Over 10 years later, clinicians are faced with the dilemma if these results still apply in centres with very high incidence of methicillin-resistant staphylococci and ampicillin resistant enterococci. It has been suggested that in centres with high rate of MRSA or other resistant Gram-positive, empirical therapy might be tailored accordingly [115, 116]. However, the benefit of early treatment of VRE infections has been debated. Preliminary single-centre experiences indicate that early use of adequate antibiotics in case of VRE infection might not have any significant impact on survival (see the paragraph on enterococci) [40, 41]. Therefore,

numerous guidelines agree that routine use of vancomycin should be avoided and indications for empirical therapy directed against Gram-positives remain in patients with presentation suggestive of a possible Gram-positive infection, such as presence of skin or soft tissue infection, pneumonia, or preliminary result of cultures positive for Gram-positive cocci [58, 115–118].

20.7.2 Targeted Therapy of Gram-Positive Infections

As a general rule, antibiotic-resistant pathogens are more difficult to treat than susceptible ones. This is true for any kind of pathogen, including Gram-positive cocci. However, for the time being, antibiotic resistance, shortage of new drugs and drug-related toxicity concern primarily multidrug resistant Gram-negative rods. For the Gram-positives, luckily, several drugs already exist or are being developed and approved for the treatment of methicillin-resistant staphylococci, such as linezolid, daptomycin, tigecycline, quinopristin–dalfopristin, telavancin, ceftaroline, ceftobiprole, tedizolid or dalbavancin. However, none of these drugs has been approved, or even properly studied in transplant recipients or in empirical therapy of febrile neutropenia. This is actually an indication which is not considered any more by regulatory agencies, and, therefore, is not searched any more by drug companies. Fewer treatment options are available against VRE, since cephalosporins are intrinsically not active against these pathogens and the only options remain oxazolidinones and daptomycin (see dedicated paragraphs).

A detailed description of all older and newer agents is beyond the aim of this chapter, and several reviews have been published on this issue [49, 119, 120]. In the following lines we try to discuss the main indications and limitation of each agent, focusing in particular, when possible, on transplant setting. The standard doses of anti-Gram-positive agents and main characteristics are reported in Table 20-3. Treatment of infections due to Gram-positive bacteria in transplant recipients is reported in Table 20-4.

20.7.2.1 Vancomycin

Vancomycin is the mainstay of therapy against penicillin- or methicillin-resistant Gram-positive cocci, but its efficacy even in vancomycin in vitro-susceptible organisms has been challenged in the following situations: (1) infections due to enterococci and staphylococci displaying increased minimum inhibitory concentration (MIC ≥2 for MRSA and ≥4 for coagulase-negative staphylococci and enterococci); (2) poor penetration into biofilm which may result in suboptimal outcomes in CVC-associated infections; (3) low and sometimes insufficient lung penetration, with suboptimal response in case of pneumonia. Additionally, nephrotoxicity and ototoxicity might represent other pitfalls for vancomycin, thus giving good reasons for choosing other agents.

The phenomenon of the low-level vancomycin resistance is one of the most common forms of decreased activity of glycopeptides and is thought to be a part of the so-called "MIC creep" phenomenon. Heteroresistant vancomycin-intermediate *S. aureus* (hVISA) strains have been identified, although vancomycin-resistant *S. aureus* (VRSA) remains very rare.

The question is whether or not pathogens expressing increased MICs to vancomycin still respond to vancomycin therapy in the real life. The issue is controversial. Increased treatment failures with higher MIC values have been reported in many, but not all studies [121–124]. A response has been to increase dosages, targeting high vancomycin trough concentrations (e.g. 15–20 μg/mL) through therapeutic drug monitoring. This strategy has been associated with modest improvements in clinical outcomes of selected patients, and is probably insufficient for achieving a desired PK/PD target (i.e. area under concentration-time curve AUC/MIC ratios ≥400 if MIC is 2 μg/mL) in severe MRSA infections. In addition, keeping through concentrations around 20 μg/ml has been associated with higher rates of nephrotoxicity, which may affect up to 20–30 % of the patients [125–127]. Last but not least, clinical outcomes in MRSA infections might depend heavily on the bacterial load at the site of infection, which appears to be worse in case of pneumonia [81, 128]. Therefore, although a number of questions remain unanswered, treatment alternative to vancomycin should be considered for infections with an MRSA isolate with vancomycin MIC ≥2 μg/mL [124] and in staphylococcal pneumonia. Other limitations of vancomycin include a large gap existing between inhibitory and bactericidal concentrations in vitro, its slow bactericidal activity and its insufficient activity in case of methicillin-susceptible staphylococci, in which oxacillin, nafcillin or cefazolin remain the drugs of choice [129].

20.7.2.2 Daptomycin

Daptomycin is a lipopeptide with a concentration-dependent bactericidal activity. The drug can be administered once-daily as a rapid (30 min) intravenous infusion. As expected for a drug with a concentration-dependent antibacterial activity, dosages higher than the approved 4–6 mg/kg/day have been associated with improved outcomes and a decreased risk of selecting for resistant strains. Therefore, in a setting with a high bacterial burden or with sequestered foci of infection, where it might be difficult to achieve adequate local antibiotic concentrations, as it might happen in valve vegetations and abscesses, higher doses might be considered [130]. In addition, in patients with a sepsis syndrome the volume of distribution is usually much higher and renal clearance increased, both good reasons for using higher doses of daptomycin. In fact, even IDSA guidelines for MRSA infections state that high-dose daptomycin, 8–10 mg/

TABLE 20-3. Dosage and main characteristics of antibiotics used in infections due to Gram-positive bacteria

Drug	Route of administration	Usual daily adult dosage	Number of daily doses	Dosing adjustment	Advantages	Disadvantages
Amoxicillin–clavulanate or ampicillin–sulbactam	i.v. or oral	i.v. 8000–12,000 mg (as amoxicillin/ampicillin)	3	Renal	Inexpensive; bactericidal; oral formulation with good bioavailability	Inactive against MRSA; diarrhoea as frequent adverse effect in case of oral administration; possible mild hepatotoxicity linked to clavulanate
Cefazolin	i.v.	6000 mg	3	Renal	Inexpensive; bactericidal; no significant interactions	Inactive against MRSA and enterococci
Ceftaroline	i.v.	1200 mg	2	Renal	Active against MRSA; bactericidal; in vitro activity against VISA, hVISA and VRSA; approved for skin infections due to MRSA	Limited clinical experience; expensive; not approved for nosocomial pneumonia; cross-allergenicity with other cephalosporins
Ceftobiprole	i.v.	1500 mg	3	Renal	Active against MRSA; bactericidal; approved for CAP and HAP caused by Gram-positive pathogens such as penicillin-resistant, *Streptococcus pneumoniae* and MRSA	Limited clinical experience; in 2015 approved only in Europe; expensive; not approved for VAP; gastrointestinal side-effects as the most frequent adverse events; cross-allergenicity with other cephalosporins
Dalbavancin	i.v.	1000 mg once followed 1 week after by 500 mg	–	Renal hepatic	Active against MRSA; long-acting antimicrobial activity; does not prolong QT interval	In 2015 approved only in the USA; approved only for acute skin and skin structure infections; impossible to rapidly discontinue treatment in case of toxicity
Daptomycin	i.v.	8 mg/kg (probably higher in case of VRE)	1	Renal	Rapidly bactericidal; active against MRSA and VRE	Inactivated by pulmonary surfactant; increases in serum CPK, rarely rhabdomyolysis; reports of drug-induced eosinophilic pneumonia; expensive
Linezolid	i.v. or oral	1200 mg	2	None	Highly bioavailable oral formulation (100%); good lung penetration; active against MRSA and VRE	Bacteriostatic; possible duration-dependent severe adverse events (thrombocytopenia, anaemia, lactic acidosis, optic and peripheral neuropathy); possible serotoninergic syndrome if co-administered with SSRI
Oritavancin	i.v.	1000 mg	1	None	Active against MRSA and VRE; extensive tissue distribution; bactericidal; once-daily dosing; no reported nephrotoxicity	In 2015 approved only in the USA; approved only for skin and skin structure infections; limited clinical experience
Oxacillin	i.v.	12000 mg	6	In the case of severe hepatic or renal failure	Inexpensive; bactericidal; no significant interactions	Inactive against MRSA and enterococci; high number of daily doses; possible cutaneous adverse events

Teicoplanin	i.v. or i.m.	600–1200 mg (a loading dose of 600 mg bid on first day of treatment)	1 (2 on the first day of treatment)	Renal	Inexpensive; red neck syndrome less common than with vancomycin	Frequently underdosed for severe infections; nephrotoxicity in case of correct dosing; possible thrombocytopenia; not available in the USA
Tedizolid	i.v. or oral	200 mg	1	None	Highly bioavailable oral formulation (100 %); good lung penetration; active against MRSA and VRE; enhanced in vitro activity compared with linezolid, and possibly less toxicity	In 2015 approved only in the USA; bacteriostatic; limited clinical experience; expensive
Telavancin	i.v.	750 mg (10 mg/kg/die)	1	Renal	Active against MRSA; approved for the treatment of HAP and VAP due to MSSA and MRSA	Inactive against VRE; Nephrotoxicity; potentially teratogenic; administered with cyclodextrin that may accumulate in the case of renal dysfunction; possible QTc prolongation, interference with laboratory coagulation tests; dysgeusia and nausea as frequent adverse events
Tigecycline	i.v.	100 mg loading dose, then 50 mg bid	2	In the case of severe hepatic failure	Active against VRE and MRSA; reported activity against *Clostridium difficile*; high volume of distribution	Bacteriostatic; low serum and lung concentrations; nausea and vomiting as frequent adverse events; FDA safety warning of higher risk of mortality than comparator agents in meta-analyses; expensive
Trimethoprim–sulfamethoxazole	i.v. or oral	15 mg/kg (dosing based on the trimethoprim component)	3–4	Renal	Inexpensive; oral formulation with good bioavailability (85 %)	Possible dermatologic and haematologic adverse effects; rarely drug-induced hepatitis; possible nephrotoxicity; interactions with ACE inhibitors or angiotensin receptor blockade possibly leading to adverse cardiac effects
Vancomycin	i.v.	2000 (30 mg/kg/die)	2	Renal	Large worldwide clinical experience; inexpensive	Nephrotoxicity; “slow” bactericidal activity; inactive against VISA, hVISA, and VRSA; increased MIC values within the susceptibility range associated with poor outcomes; possible red neck syndrome

HAP hospital-acquired pneumonia, *MRSA* methicillin-resistant *Staphylococcus aureus*, *MSSA* methicillin-susceptible *Staphylococcus aureus*, *VRE* vancomycin-resistant CAP, community-acquired pneumonia; HAP, hospital-acquired pneumonia; hVISA, heterogenous vancomycin-intermediate Staphylococcus aureus; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant Staphylococcus aureus; MSSA, methicillin-susceptible Staphylococcus aureus; SSRI, selective serotonine reuptake inhibitor; VAP, ventilator-associated pneumonia; VISA, vancomycin-intermediate Staphylococcus aureus; VRE, vancomycin-resistant enterococci.

TABLE 20-4. Treatment of infections due to Gram-positive bacteria in transplant recipients

Pathogen	First line	Alternative	Comments
Corynebacterium urealyticum	Vancomycin	Linezolid, daptomycin	Aminoglycoside might be added to vancomycin for better efficacy, but high risk of nephrotoxicity
Corynebacterium urealyticum	Vancomycin	Linezolid, daptomycin	
Enterococci			
Ampicillin-susceptible	Ampicillin	Ampicillin + ceftriaxone in case of gentamycin resistant endocarditis Linezolid Daptomycin	If susceptible gentamycin can be associated for bactericidal activity; if susceptible nitrofurantoin may be used for urinary tract infections only
Ampicillin-resistant	Vancomycin	Daptomycin Linezolid	If susceptible gentamycin can be associated for bactericidal activity, but high risk of nephrotoxicity; if susceptible nitrofurantoin may be used for urinary tract infections only
VRE	Daptomycin Linezolid		
Listeria monocytogenes	Ampicillin	Meropenem Trimethoprim–sulfamethoxazole Linezolid	Gentamycin should be added in case of meningoencephalitis
Nocardia	TMP–SMX + meropenem–imipenem	Meropenem Linezolid ± meropenem Imipenem + amikacin	Prolonged treatment recommended
Pneumococci	Penicillin Ampicillin Ceftriaxone	Vancomycin	
Rhodococcus equi	2 of the following: levofloxacin/ciprofloxacin or rifampicin or azithromycin	Imipenem or vancomycin + one of first line drugs	Prolonged treatment recommended
Staphylococci			
Methicillin-susceptible staphylococci	Oxacillin Nafcillin Cefazolin	Amoxicillin–clavulanate Ceftriaxone Daptomycin	Outcomes with vancomycin has been reported inferior to anti-staphylococcal penicillin
Methicillin-resistant staphylococci	Vancomycin	Daptomycin Linezolid Telavancin Anti-MRSA cephalosporin	Vancomycin should be avoided in case of high MIC values No data for new cephalosporins
Viridans streptococci	Penicillin Ampicillin	Vancomycin Piperacillin–tazobactam Meropenem Cefepime	

TMP–SMX trimethoprim–sulfamethoxazole.

kg/day, might be used for complicated bacteraemia or endocarditis [24].

Concerns that higher dosages of daptomycin may be associated with muscular toxicity have been raised. A reassuring answer has been provided by the EU-CORE study, which is a retrospective, multicentre, non-interventional, manufacturer-funded patient registry designed to collect real-world data on the use of daptomycin in 18 countries worldwide between 2006 and 2012 [131]. In this database, 6075 patients were included and 1097 received high dose daptomycin (>6 mg/kg), mainly for osteomyelitis, foreign body prosthetic infections, and endocarditis. Increasing the dose of daptomycin was not related to any increase in toxicity, including muscle toxicity and creatine phosphokinase elevation [130, 131]. Rapid bactericidal activity and good biofilm penetration make daptomycin a valid choice in case bacteraemia and CVC-associated infection. Once-daily administration is particularly suitable for outpatient administration, which in turn might allow early hospital discharge. The main limitation of daptomycin, both in general and in the transplant population, is its lack of activity in pneumonia. Indeed, in the lungs the drug is inactivated by alveolar surfactant. In this case the safety and efficacy of daptomycin plus linezolid combination has been suggested.

Data from the aforementioned retrospective observational study (EU-CORE) obtained from neutropenic patients

showed it was a useful and well tolerated treatment [132]. Daptomycin was administered mainly in case of bacteraemia (78 %), and VRE infection (47 %), and discontinuation due to a possible adverse event occurred in 6 % of patients [132].

20.7.2.3 *Daptomycin in VRE Infections*

The optimal therapeutic choice in VRE infections remain to be established. At present, linezolid is the only drug approved for this indication, although clinical experience with the use of daptomycin is growing. Three recent meta-analyses addressed the comparison of outcomes of VRE BSI treated with linezolid and daptomycin [45–47]. All of them concluded that the quality of the analysed data was poor, since most of the data were coming from single-centre retrospective experiences. With the evident limit of the low quality of data, the mortality rates were found slightly higher in case of daptomycin, compared to linezolid [45–47]. On the contrary, a recent retrospective cohort study comparing linezolid and daptomycin for the treatment of VRE-BSI in 644 patients admitted during years 2004–2013 concluded the opposite, i.e. that linezolid was associated with slight but significantly higher risk of failure compared to daptomycin (adjusted risk ratio, 1.15; 95 % CI 1.02–1.30; $p = 0.026$) [48]. Additionally, linezolid was also associated with increased 30-day mortality and a higher rate of microbiologic failure. Indeed, the reason for the discrepancy between the above mentioned studies might be at least partially ascribed to the dosing of daptomycin, which remains problematic in enterococcal infections. According to in vitro data, the optimal dose of daptomycin against enterococci should be 10–12 mg/kg/day, which is almost twofold higher than approved dose, in order to improve efficacy and prevent the emergence of resistance [133]. In our opinion, neither linezolid nor daptomycin is an ideal treatment option in this setting. Linezolid is bacteriostatic, may offer suboptimal activity in deep-seated infections, and, in case of prolonged use, may be associated with significant haematological toxicity. The limitations of daptomycin in VRE infections include emergence of resistance during therapy, the presence of mutations associated with resistance even in isolates that are reported as susceptible in vitro but with higher MIC values, the lack of data on the optimal dose and its lack of activity in pneumonia [134].

In conclusion, enterococci are increasingly frequent in HSCT and SOT settings. *E. faecium* is growing in incidence, but resistance to vancomycin varies significantly by geographical regions. Enterococcal infections, both due to VRE and vancomycin-susceptible *E. faecium*, could be regarded as a marker of poor clinical status and important comorbidities [34]. Optimal treatment options remain yet to be defined.

20.7.2.4 *Oxazolidinones (Linezolid and Tedizolid)*

Linezolid is the first oxazolidinone approved in 2000 in the USA and a year later in Europe for both intravenous and oral use. It is bacteriostatic, with good activity against many Gram-positive aerobic bacteria, including resistant strains of several species, such as MRSA, penicillin-resistant pneumococci and VRE. It is also active against less frequently isolated strains, like *Corynebacterium* spp., *Listeria monocytogenes, Bacillus* spp., *Rhodococcus equi, Nocardia* spp. and *Lactobacillus* spp [120]. It exerts activity against resistant strains of *M. tuberculosis*. Its main features are good tissue and organ penetration, including lung epithelial lining fluid, central nervous system and eyes; and its excellent oral bioavailability. No dose adjustment is necessary in renal impairment, which makes it a suitable treatment option in patients with renal failure and if other nephrotoxic medications are being used.

Adverse events are unfortunately a pitfall for this drug, because of its myelotoxocity, which affects mostly red cell and platelets lineages, risk of lactic acidosis, and peripheral or optic neuropathy. All these side effects are seen also after relatively short treatment periods (as short as 14 days). Fortunately, all side effects, except for neuropathy which can be irreversible, subside shortly after drug discontinuation [120]. Transplant recipients frequently receive multiple other concomitant therapies, including anti-depressive medications. Therefore, it is worth mentioning that linezolid is a mild monoamine oxidase inhibitor and serotonin syndrome has been reported in patients taking selective serotonin reuptake inhibitors (SSRI). Data from retrospective studies suggest that co-administration is not contraindicated as long as such patients are closely monitored for the development of the serotonin syndrome [135].

Some experiences on the use of linezolid in HSCT and neutropenic patients have been published. A randomised double-blind study compared linezolid to vancomycin in over 600 febrile neutropenic patients. This trial was performed, despite previous trials (namely the EORTC study) had shown that vancomycin was not useful for this indication. Although the study design was erroneous (it should have been a placebo-controlled trial of linezolid vs. nothing) a similar efficacy rate was reported for both groups. However, a delayed recovery of absolute neutrophil counts was present in the modified intention to treat population receiving linezolid, while no difference in time to platelet engraftment was observed [136]. On the contrary, in a small single-centre experience, haematologic side effects, which are particularly important in HSCT recipients, have not been reported significantly more frequently in patients treated with linezolid during the pre-engraftment phase [137]. In particular, time to neutrophil and platelet engraftment were not different in 33 cases who received more than 7 days of linezolid treatment compared to controls [137]. Additionally, linezolid was reported safe and effective in 46 adult liver transplant recipi-

ents, with no cases of thrombocytopenia occurred during treatment for over 2 weeks [138].

After 15 years of use, resistance to linezolid has been rarely seen [120]. Resistance mechanisms were first described for *E. faecium* and *S. aureus*, and later also for *E. faecalis*, but they affect less than 1 % of strains, as documented in a surveillance study, which reported non-susceptibility rates between 0.03 and 1.83 % when tested against more than 42,419 clinical isolates of staphylococci and enterococci across nine consecutive surveillance years (2004–2012) in the USA [139]. Cases of linezolid-resistant isolates of staphylococci are described especially after prolonged therapy (>21 days) [140]. Resistance of enterococci to linezolid has been associated with previous linezolid therapy, although nosocomial acquisition of resistant enterococci has been also reported [120, 141, 142]. Coagulase-negative staphylococcal isolates, mainly *S. epidermidis*, currently account for the majority of Gram-positive organisms displaying elevated MIC to linezolid [139].

Tedizolid, a new oxazolidinone, has been recently approved for skin and soft tissue infections [143]. While sharing linezolid's excellent bioavailability and tissue penetration, its advantages include once daily dosing (200 mg/die) and fewer side effects, mostly gastrointestinal and haematological, together with the lack of interactions with SSRIs.

20.7.2.5 Tigecycline

Tigecycline is the first in a new class of glycylcyclines (similar to tetracyclines), that has been approved for the treatment of complicated skin and skin structure and intra-abdominal infections in adults. Although it is active against MRSA, it is not recommended for this indication mainly because of the 2010 FDA's warning, based on the observation from pooled efficacy data of an increased overall mortality among patients treated with tigecycline for serious infections. This increased mortality was probably the effect of study designs which did not take into adequate consideration the lack of activity of tigecycline against *P. aeruginosa.* Two meta-analyses confirmed subsequently the data [24, 144, 145]. In addition, tigecycline should not be used in patients with MRSA bacteraemia due to low plasma concentrations and bacteriostatic activity. At present, tigecycline is mainly used in combination regimens for the treatment carbapenemase-producing *Klebsiella pneumoniae* or for intra-abdominal infections.

20.7.2.6 New Anti-MRSA Cephalosporins

New anti-MRSA cephalosporins such as ceftaroline or ceftobiprole are active against methicillin-resistant staphylococci and share common characteristics of beta-lactams, namely bactericidal activity, excellent safety profile and possible paediatric administration. Although developed as anti-MRSA drug, ceftaroline in not recommended for treatment of MRSA pneumonia due to the lack of data because, surprisingly, few or no patient with this infection were included in the registration trial. Their drawbacks include lack of oral formulation, need to administer two to three times daily, no meaningful activity against enterococci and, last but not least, lack of data in immunocompromised and transplant patients.

20.7.2.7 New Lipoglycopeptides

New lipoglycopeptides such as telavancin, dalbavancin and oritavancin are now available to treat MRSA infections. They are all approved for immunocompetent patients, and dalbavancin and oritavancin offer an advantage for outpatient therapy due to their long half-life and thus the possibility of being administered up to every 7–10 days. Their role in transplant recipients remains yet to be established.

20.8 Conclusion

In conclusion, Gram-positive micro-organisms remain dangerous pathogens, both for HSCT and SOT recipients. They are observed relatively early after transplant in both cases, although they are related to different predisposing conditions, such as neutropenia in HSCT and mechanical defence impairment related to surgery and ICU stay in SOT. They can cause either bacteraemias or localised infections in both cases, even though bacteraemias and pneumonia are more frequent in HSCT recipients while abscesses and intra-abdominal infections, particularly enterococcal, are prevalent in the SOT population (especially in case of liver or pancreas transplants). Staphylococcal pneumonia can be an issue in thoracic organ transplantation. The mortality rate is variable, and usually lower than in Gram-negative or fungal infections. Enterococcal infections, especially VRE, can be a marker of disease severity. In late post-transplant phases, characterised by the risk of infections due to encapsulated organisms, pneumococci are the most important and vaccination seems the most effective strategy. Although widely used, an empiric anti-Gram positive coverage should not be the rule in febrile neutropenic patients undergoing transplantation, unless specific risk factors are present. Antibiotic resistance is not infrequent in Gram-positive bacteria, with high rate of methicillin resistance. VRE are still relatively rare in Europe and more common in the USA, and their treatment remains challenging. Fortunately, and contrary to Gram-negative rods, new drugs are available for treating resistant Gram-positive infections. Although no drug can be defined as ideal, several treatment options exist.

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21 Gram-Negative Bacterial Infections After Hematopoietic Stem Cell or Solid Organ Transplantation

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21.1 Introduction

21.1.1 Epidemiology of Gram-Negative Rods Infections

Gram-negative rods (GNR) cause significant morbidity and mortality in hematopoietic stem cell (HSCT) and solid organ transplant recipients (SOTR) [1–7]. These patients are prone to infection with GNR as a result of neutropenia, mucositis, the use of invasive devices and due to operation in SOTR [8]. Invasive GNR infections usually arise from abdomen (including infections of the hepatobiliary system in liver transplant recipients) [9], the urinary tract (especially occurring in renal transplant recipients) and lungs (occurring in all transplant groups, but notably in lung transplant recipients). In SOTR, complications (for example, portal vein thrombosis in liver transplant recipients) and prolonged mechanical ventilation represent significant risks. Risk factors for invasive GNR infection in neutropenic patients include age >45 years, recent administration of beta-lactams, chills, urinary symptoms, absence of gut decontamination with both colimycin and aminoglycosides [10] and previous colonization [11]. In the early years of transplantation, GNR were the leading cause of serious bacterial infection in both bone marrow and SOT recipients [12]. Later, gram-positive pathogens have become more frequent [13, 14]. Reemergence of GNR is reported in recent years [15–18]. A recent review of studies published during 2005–2011, on bacterial infections in patients with hematological malignancies or post HSCT, reported gram-positive to GNR ratios in adults 60 %:40 %, with a huge variation between centers, from 85 %:15 % to 26 %:74 % [17]. The corresponding numbers in children were: 58 %:42 %, ranging from 86 %:14 % to 32 %:68 % in individual studies. The main GN pathogens causing bacteremia in HSCT recipients (expressed as median prevalence, with range) were *Enterobacteriaceae* (24 %, 6–54 %), followed by *Pseudomonas aeruginosa* (10 %, 0–30 %) [17]. An ECIL-4 survey performed in 2011 on surveillance of bacteremia in hematology and HSCT patients summarized recent data from 39 European centers. As compared to published data, it showed a slight reduction of the gram-positive to GNR ratio (55 %:45 % vs. 60 %:40 %) and an increased rates of Enterobacteriaceae (30 % vs. 24 %), and decreased rate of *Pseudomonas aeruginosa* (5 % vs. 10 %) [17].

GNR are important cause of infections in SOTR [19]. 15.4 % of 956 SOTR developed GNR infection in one study [20]. The unadjusted overall incidence of gram-negative BSI was 15.8/1000 person-years following SOT [21]. In a recent multicenter Italian study, recipients of either heart or lung graft were at the highest risk to develop GNR bacteremia [20]. In another study, however, the rate of GNR infections was highest in simultaneous kidney–pancreas (40/1000 person/years) and lowest in liver and heart (12/1000 person years) recipients [21]. Others reported that 50–60 % of all BSI in liver Tx patients were due to GNR [7, 22]. The majority of infections with the GNR in transplant recipients occur in the early posttransplant period, especially in the first month post transplantation [9, 15, 16, 20, 21, 23].

21.1.2 Clinical Manifestations and Outcome

GNR infections may present with diverse clinical pictures: bacteremia with or without concomitant local site infections. One study reported pulmonary infections in 28.4 %, urinary tract infections in 14.8 %, and skin or soft tissue infections in 9.7 % [24]. Other studies have reported that septic shock was specifically associated with infection with GNR [25] or with drug-resistant GNR infections [26].

Infection with GNR is associated with worse prognosis. Mortality rate in HSCT patients experiencing GNR BSI was 59 % in one study [27]. In other studies, 7-and 30-day mortality after BSI onset was 17–22 % and 24–31 % [23, 28]. In one study, among aerobic gram-negative pathogens, *Pseudomonas aeruginosa* had the highest associated mortality rate (40 %) followed by the *Enterobacter, Citrobacter, Serratia* group with 25 % mortality and *E. coli* or *Klebsiella* with 3 % mortality within a 7-day period [15]. The overall unadjusted 28-day all-cause mortality of GNR BSI was

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4.9 % in SOTR and was lower in kidney than in liver recipients (1.6 % vs. 13.2 %, $p < 0.001$) [21]. However, another study reported lower mortality 2/70 (3 %) patients [29].

21.1.3 Antimicrobial Resistance

21.1.3.1 Definitions

The isolate is considered multidrug-resistant (MDR) if it is non-susceptible to at least one agent in ≥3 therapeutically relevant antimicrobial categories; extensively drug-resistant (XDR) was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e., bacterial isolates remain susceptible to only one or two categories); and pandrug-resistant (PDR) was defined as non-susceptibility to all agents in all antimicrobial categories [30].

21.1.3.2 Mechanisms of Resistance

The major mechanism of resistance to cephalosporins is beta-lactamase production. The most important beta-lactamases are the plasmid-mediated extended-spectrum beta-lactamases (ESBLs), including CTX-M, TEM and SHV and inducible group 1 AmpC cephalosporinases, which are resistant to beta-lactamase inhibitors [31–39]. Class B beta-lactamases (metallo beta lactamases, MBLs) hydrolyze all penicillins, cephalosporins, and carbapenems, with the exception of monobactam aztreonam. The most common types of MBL are IMP and VIM groups [40].

The usual mechanism of resistance to quinolones is mutation of the genes that encode the target enzymes (DNA gyrase and topoisomerase IV) for quinolones.

21.1.3.3 Epidemiology of Resistance

There is a growing problem of increasing resistance to antibiotics all over the world, including in oncological and transplant patients. There is a significant site-to-site variation in the epidemiology of resistance. Prevalence of resistance is influenced by the local policy of antibiotic use for prophylaxis and treatment, infectious control measures, as well as prevalence of resistance in the whole hospital and country.

Recent literature review of studies which report on the epidemiology of BSI in HSCT patients reported that 41 % (range 18–74 %) of GNR bacteria are resistant to fluoroquinolones, 28 % (6–41 %) to aminoglycosides, 43 % (17–45 %) to ceftazidime and 20 % (11–72 %) to carbapenems [17].

According to the ECIL-4 questionnaire assessing the recent situation in HSCT centers in Europe, median rates of ESBL-producers among *Enterobacteriaceae* was 15–24 %, aminoglycoside-resistant GNRs 5–14 % and carbapenem-resistant *Pseudomonas aeruginosa* 5–14 %. Resistance rates were significantly higher in South-East vs. North-West European HSCT centers [17]. The resistant pathogens causing most clinical problems were reported to be ESBL-producing *Enterobacteriaceae* in 28 (76 %) of centers, whilst the next-most frequent concerns were fluoroquinolone-resistant GNRs, ($n = 17$, 46 %), carbapenem-resistant *Pseudomonas aeruginosa* ($n = 15$, 41 %) and much less multidrug-resistant (MDR) *Acinetobacter baumannii* ($n = 5$, 14 %).

Several studies report on increase in MDR GNR rods in HSCT patients, including ESBL-producing *Enterobacteriaceae*, AmpC cephalosporinase hyperproducing *Enterobacteriaceae*, MDR *P. aeruginosa, Stenotrophomonas maltophilia*, and *Acinetobacter baumannii* [18, 41–43].

21.1.3.4 Risk Factors for Resistance

The most important risk factor for infection with resistant pathogens is prior colonization or infection by resistant organisms such as ESBL- and carbapenemase-producing Enterobacteriaceae, colistin-resistant *Klebsiella pneumoniae*; resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia* [44–56].

Another important risk factor for infection with resistant GN in transplant patients is previous exposure to broad spectrum antibiotics for treatment or prophylaxis [18, 40, 42, 57–66]. Especially important in this context is the potential role of fluoroquinolone prophylaxis in HSCT recipients [16, 60, 67–72].

Treatment with third-generation cephalosporins was associated with infection due to MDR GNR pathogens [73].

Other risk factors for resistant GNR pathogens in HSCT patients include serious illness (e.g., end-stage disease, sepsis, pneumonia), nosocomial infection, prolonged hospital stay and/or repeated hospitalizations, intensive care unit (ICU) stay, urinary catheters, and older age [18, 42, 45, 46, 48–55, 60, 61, 70–75].

In SOTR, risk factors for infection with resistant GNR include nosocomial acquisition, longer hospital stay, admission to hospital for more than 48 h before transplantation, previous transplantation, prior ICU admission, septic shock, age greater than 50 years, HCV infection, devices, increased severity of the underlying disease, renal failure with or without dialysis [20, 26, 62, 64, 66, 76–78].

In renal Tx recipients, risk factors for resistant GNR infections were double kidney–pancreas transplantation, requirement for posttransplant hemodialysis, surgical reoperation, posttransplant urinary obstruction, and requirement for nephrostomy [64, 77]. Lung transplant recipients had a higher risk for isolation of carbapenem-resistant bacteria in one study [20].

21.1.3.5 Impact of Resistance

Infections caused by resistant GNR, including ESBL-producing Enterobacteriaceae, MDR NFGNR, carbapenem-resistant GN, are associated with increased mortality in both HSCT and SOT patients [7, 22, 41, 42, 61, 64, 79–85].

Many of these studies show that failure to cover GNR pathogens, particularly ESBL producers, MDR *P. aeruginosa*, and CRE in empiric treatments significantly and independently impairs outcomes patients, increasing mortality and prolonging hospitalization [43, 60, 61, 71, 72, 80, 86–89]. Infection with multiresistant bacteria was associated with graft loss in kidney transplant recipients [64].

21.1.4 Treatment

Serious infections due to the GNR rods in transplant recipients should be managed with a beta-lactam or quinolone antibiotic, active in vitro against the infecting organism.

Several studies demonstrate that onco-hematological and transplant patients infected with resistant and MDR GNR are significantly more likely to receive an inadequate initial empiric antibiotic therapy than those with a susceptible strain [18, 26, 42, 60, 61, 71]. These studies also show that the time to appropriate therapy is much longer where the pathogen is resistant.

21.2 Enterobacteriaceae

21.2.1 Epidemiology

The members of the *Enterobacteriaceae* are GNR bacilli which are usually resident in the gastrointestinal tract. Examples of such organisms include *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae, Proteus mirabilis*, and *Citrobacter freundii*. The majority of infections are caused by *E. coli*, followed by *Klebsiella* spp. and *Enterobacter*/*Citrobacter*/*Serratia* spp. [15, 21].

The majority of BSI in SOTR are due to *Enterobacteriaceae* [7, 20–22], they mainly occur during the first month after SOT [7, 22]. The risk is highest in transplant recipients whose peritoneal cavity has been breached (liver, intestinal, and pancreatic transplant recipients). Spillage of enteric organisms into the peritoneal cavity in such patients may lead to intra-abdominal abscess formation and manipulation of the biliary tree may lead to cholangitis.

Pneumonia occurring early after lung transplantation and urinary tract infections in renal transplant recipients may be more likely to be due to the *Enterobacteriaceae* [90, 91].

21.2.2 Clinical Manifestations and Outcome

Infections with the *Enterobacteriaceae* in transplant recipients have a multitude of clinical presentations. The sites of infection have been diverse and have included the urinary tract, lower respiratory tract, intra-abdominal, bloodstream, and wounds.

All transplant recipients, by virtue of prolonged hospitalization, may develop skin and upper respiratory tract colonization with gastrointestinal tract flora. Therefore, central venous line related infections and ventilator-associated pneumonia may occur due to the *Enterobacteriaceae*. Liver transplant recipients are prone to development of intra-abdominal infections with the *Enterobacteriaceae*. These may be mixed infections with anaerobes and enterococci. Examples of such infections include peritonitis, intra-abdominal abscess, cholangitis, and infected bilomas. Renal transplant recipients may develop complicated urinary tract infections or develop secondary infections within urinary leaks. The most common infections with the *Enterobacteriaceae* in lung and heart transplant recipients are pulmonary infections, which occur in all other SOT [92].

Death in neutropenic or other heavily immunosuppressed patients may occur within hours of onset of signs of infection, in the absence of appropriate supportive and antibiotic treatment. *Enterobacter* bacteremia was associated with 63 % mortality rate in one SOTR study [12].

21.2.3 Antimicrobial Resistance

21.2.3.1 Mechanisms of Resistance

The increasing resistance to carbapenems in *Enterobacteriaceae*, especially but not limited to *K. pneumoniae*, is a major concern. Resistance to carbapenems may be mediated by several mechanisms, including production of carbapenemases, efflux pump, decreased membrane permeability, and combination of these mechanisms [93]. Combination of plasmid-encoded AmpC or ESBL expression together with decreased cell membrane permeability may be also responsible for resistance to carbapenems [94]. The main mechanism of carbapenem resistance in *Enterobacteriaceae* in most parts in the world is hydrolysis by the serine class A β-lactamase *Klebsiella pneumoniae* carbapenemase (KPC). This mechanism also conferred resistance to all cephalosporins, aztreonam, and beta-lactamase inhibitors such as clavulanic acid and tazobactam. The gene encoding this enzyme *bla*KPC is located on plasmids and can be transferred between different species [94, 95]. Specifically, KPC-producing *Klebsiella pneumoniae* clone, sequence type ST258, has emerged and disseminated worldwide, being responsible for several outbreaks, including in HSCT patients [96–99].

Since 2009, a novel plasmid-encoded enzyme, New Delhi MBL (NDM), has spread through India, Pakistan, and the UK, and was reported also in transplant patients [100, 101]. These strains typically also have 16S rRNA methylases, conferring complete aminoglycoside resistance [102].

Carbapenem- resistant *Enterobacteriacea* (CRE) are frequently resistant to other antibiotics, including those considered as a “last resource,” as colistin, tigecycline, fosfomycin, and aminoglycosides.

21.2.3.2 Epidemiology of Resistance

Two to 44 % of *Enterobacteriaceae* in HSCT patients are ESBL producers [15, 16, 41, 73, 79, 81, 83, 103]. There is significant increase in carbapenem-resistant GNRs, especially *Klebsiella pneumoniae* (CRKp), in some HSCT centers [104].

In one study, CRKp infection was independently associated with recent stem-cell transplantation or organ, and it found to be associated with numerous health care-related risk factors and with high mortality [105].

In a recent retrospective Italian survey, more than a half of 52 centers reported on CRKp infections, and the incidence is growing, especially in allogeneic HSCT patients [43]. The incidence of CRKp infections was 0.4 % (from 0.1 % in 2010 to 0.7 % in 2013) in auto-SCT and 2 % (from 0.4 % in 2010 to 2.9 % in 2013) in allo-SCT populations [43].

ESBL producing organisms frequently colonize the gastrointestinal tract of SOTR.

The rate of ESBL-producers among Enterobacteriaceae in SOTR is 8–77 % [21, 22, 29, 77, 106–111]. 42.3 % of 80 MDR GNR strains isolated from 350 SOT recipients were ESBL-positive (mainly *Enterobacteriaceae*) [39].

There is increasing quinolones resistance of GNR bacteria in SOT [21, 112]. One study reported on increasing resistance among *Escherichia coli* isolates to fluoroquinolone antibiotics from 0 to 44 % ($p=0.002$) throughout the study period (1996–2007) [21].

Several studies reported on infections caused by carbapenem-resistant *Enterobacteriaceae* (CRE) in SOT patients. In one cohort study, organ transplant recipients appeared to be at increased risk for CRKp bacteremia [113]. Incidence of CRE infections was 1.3–12.9 % in liver, 9.4–26.3 % in kidney, 0.4–6.6 % lung, 7.5–16.7 % in heart transplant patients [100, 114]. In one recent multicenter Italian study, 26 % of all GNR bacteria and half of all *Klebsiella pneumoniae* in SOT patients were carbapenem-resistant [20]. SOTR were involved in hospital outbreak with CRE [115]. The median time since SOT to infection was 12–90 days, late infection 218 days after lung transplant was reported however [116]. The site of infection was bacteremia in 17–100 %. The other sites were pneumonia 25–50 % in liver, lung, and heart SOT; UTI in 60–100 % in renal transplants, intraabdominal (mainly in liver transplants) and soft tissue infections.

Colonization with CRKp endangers patients with subsequent infection. Generally, patient colonized with CRKp has 7.8–16 % chance to develop CRKp BSI [117, 118]. In transplant patients this risk is higher. The rates of BSI among rectal CRKp carriers was 39 % in hematological and allo-HSCT patients, 26 % in auto-HSCT, 18.8 % in SOTR, 18.5 % in ICU and 16 % in general ward patients [43, 117–119]. Patients with documented CRKp infection before allogeneic HSCT with had high chance of relapse—45.4 %; 90 % of them died despite early targeted therapy [43].

In liver transplant patients, CRKp infection rates among patients non-colonized, colonized at the time of transplantation, and colonized after transplantation were 2, 18.2, and 46.7 % in one study ($p<0.001$) [120]. In another study in liver Tx, 8/9 patients known to be colonized with KPC-2 CRKp developed infection, and five (56 %) were confirmed to have BSI with KPC-2-KP [84].

Certain factors predispose CRKp colonized patients to develop infection, such as number of colonization sites, admission to the ICU, abdominal invasive procedure, chemotherapy/radiation therapy, diabetes mellitus, solid tumor, tracheostomy, urinary catheterization, having a central venous catheter, receipt of antibiotics, renal replacement therapy; mechanical ventilation >48 h; hepatitis C virus (HCV) recurrence [117, 118, 120]. Some of these factors are routinely present in transplant patients, which can explain higher risks of invasive infection following colonization. In patients with health care-associated bacteremia, prior use of carbapenems may be only second to cephalosporins as the most significant antibiotic exposure associated with the involvement of ESBL-producing organisms [48].

21.2.3.3 Impact of Resistance

Mortality rate in infections caused by ESBL-producing bacteria was 38–52 % as compared to 5.5–29 % in infections caused by non-ESBL producing bacteria in HSCT recipients [41, 61, 79–81].

Mortality in infections caused by CRE was significantly higher as compared to non-CRE bacteria (33–72 % vs. 9–22 %) in several studies, including in transplant patients [43, 82, 104, 105, 121–123]. The infection-related mortality rates were 16 and 64.4 % in autologous and allogeneic HSCT recipients, respectively. Almost all patients died because of CRKp infection in one recent study [43]. The high rate of mortality in allo-HSCT patients was comparable or higher than that reported in previous series of intensive care unit (32–41 %), some solid organ transplant (40 %) and hematologic malignancies (65 %) patients [43, 100, 124]. The infection-related mortality rate was 48 % in patient who received CRKp- targeted 1st line therapy as compared with 73 % in those who received a not CRKp-targeted first-line antibiotic therapy ($p=0.002$) [43].

CRE bacteremia in SOTR caused septic shock in 18 % of patients, and was recurrent and persistent in 29 % each, in one study [116]. Summary of several studies in SOTR infected with CRE reported on 37 % mortality [100], in one study it reached 78 % [84]. SOTR with at least one positive culture for carbapenem-resistant GNR had a 10.23-fold higher mortality rate than those who did not [20]. Bacteremic or non-bacteremic infections due to CRKp resulted in a five-fold increased risk of death after liver transplantation [125]. Retrospective cohort study comparing SOTR with a first episode of UTI due to CRKp, ESBL-producing *Klebsiella*

pneumoniae, or susceptible *Klebsiella pneumoniae* demonstrated that CRKP is associated with long length of stay, and microbiological failure [78]. Six of 13 (46 %) kidney transplant recipients with CRKp infection, and none of the patients with carbapenem-sensitive *Klebsiella pneumoniae* infection, died within 6.5 months of infection onset [126]. Resistance to colistin has been independently associated with worse outcomes in patients infected with CRKp [127].

21.2.4 Treatment

An important caveat is that ESBL-producing organisms may appear susceptible in vitro to third generation cephalosporins (ceftazidime, cefotaxime or ceftriaxone) or cefepime, yet be functionally resistant to these agents [128]. 8–20 % of patients receiving broad-spectrum cephalosporins for *Enterobacter* infection had resistant isolates under treatment due to induction of AmpC [129–132].

ESBL producers in vitro are inhibited by beta-lactamase inhibitors (sulbactam, clavulanate, tazobactam). However, MIC to these agents rises with increasing inoculum [133]. Quinolones are usually inappropriate for treatment, as resistance to quinolone is frequent in ESBL producing bacteria: 20–90 % ESBL producers were resistant to quinolones, as compared to 2–42 % of non-ESBL-producers [61, 79, 134]. Carbapenems should be regarded as the drugs of choice for serious infections with β-lactamases-producing organisms [35, 37, 38, 121, 135].

Treatment of carbapenem-resistant GNR is discussed below. Resistance to agents active against carbapenem-resistant GNR has been reported. For example, among KPC-Kp isolates in HSCT patients, 80.8 % were susceptible to colistin, 69.2 % to tigecycline, and 65.4 % to gentamicin in one study [104].

Appropriate treatment for resistant bacteria is frequently delayed. Inadequate empirical therapy was most common in SOTR infected with ESBL bacteria [56]. CRKp-targeted therapy was provided with more than 2 days delay in one study in patients with hematological malignancies [124].

21.3 Non-fermentative Gram-Negative Rods (NFGNR)

21.3.1 Epidemiology

The NFGNR include *Pseudomonas aeruginosa, Acinetobacter* spp., *Burkholderia cepacia, Stenotrophomonas maltophilia*, and some other more rare bacteria. Non-fermentative refers to their inability to ferment glucose (instead, most species degrade glucose oxidatively). Non-fermentatives are less frequent causes of BSI than *Enterobacteriaceae* in transplant patients [7, 12, 15, 17, 20–22]. However, these are more frequent causes of pneumonia [20].

Pseudomonas aeruginosa is the most frequent of the NFGNR, causing about 5–15 % of bacteremias [7, 17, 20, 21]. *Acinetobacter* spp, *Burkholderia cepacia*, and *Stenotrophomonas maltophilia* are considerably less frequent—responsible for about 2 % (0–12 %) of bacteremia in HSCT [18, 27, 136–145] and 2–10 % of GNR bacteremia in SOTR [7, 20].

Pseudomonas aeruginosa is responsible for 8–25 % of cases of pneumonia occurring in SOTR [92, 146]. Lung transplant recipients are at greatest risk [90, 147, 148].

Between 2 and 13 % of patients with cystic fibrosis (CF) are colonized with *Burkholderia cepacia* [149–151]. Increasing age and advanced lung disease are risk factors for *Burkholderia cepacia* colonization implying that candidates for lung transplantation may also be at the highest risk of *Burkholderia cepacia*.

21.3.2 Clinical Manifestations and Outcome

In one study on NFGNR bacteremia in cancer patients (including HSCT), the risk of complications was high (47 %), including 35 % with septic syndrome, 19 % pneumonia, 3.5 % enterocolitis, and 3.5 % soft-tissue infections [139]. There are few clinical characteristics which distinguish transplant recipients with infection with NFGNR from patients with infection with the *Enterobacteriaceae*.

Pseudomonas aeruginosa bacteremia may be associated with ecthyma gangrenosum. The skin lesions of ecthyma ganrenosum may be multiple, with rapid evolution through stages of macules, nodules, vesicles, and ulcerative eschars. The lesions contain little, if any, pus. In children the lesions tend to occur on the perineum and buttocks, but they may appear anywhere. Although ecthyma gangrenosum is regarded by some as pathognomonic for *Pseudomonas aeruginosa* bacteremia, similar lesions have been reported with other etiologies of bacteremia, such as *Stenotrophomonas maltophilia* [152].

In SOT patients, *Pseudomonas* can cause pneumonia, UTI (mainly in renal Tx) and bacteremia [64, 106]. *Pseudomonas aeruginosa* may be associated with cholangitis in liver transplant recipients. De novo colonization of the lung allograft by *Pseudomonas* is associated with the subsequent development of bronchiolitis obliterans [153].

Mortality is especially high in *Pseudomonas aeruginosa* bacteremia in both HSCT and SOTR: 39–67 % [12, 15, 16, 23, 28, 62, 154]; the majority of patients died within 7 days from the onset of infection. Mortality is especially high if caused by multidrug resistant (MDR) bacteria [83, 155]. Onset of *Pseudomonas aeruginosa* blood stream infections in ICU is an independent predictor of mortality after HSCT and SOT patients [62].

Acinetobacter spp, *Stenotrophomonas maltophilia*, and other NFGNR were responsible for catheter related bacteremia, severe sepsis, severe hemorrhagic pneumonia and soft

tissue infection in HSCT patients [18, 27, 136–145] and 2–10 % of GNR bacteremia in SOTR [7, 20, 156]. The most frequent clinical manifestation of *Stenotrophomonas maltophilia* is pneumonia and the second most frequent is CVC-related bacteremia [144, 156–158]. It must be recognized that not every isolate from the respiratory system is a true cause of pneumonia, but may represent colonization of respiratory tract.

Stenotrophomonas maltophilia emerges particularly in patients with prior broad-spectrum antimicrobial therapy.

Acinetobacter spp. can cause suppurative infections in virtually every organ system; mainly they cause nosocomial infections [9]. Lung transplant recipients infected with *Acinetobacter* were less likely to clear infection as compared to non-transplant patients, and more likely to die because of *Acinetobacter* infection [85]. Infections with *Acinetobacter* can be severe, a third of them were associated with septic shock and 47.1 % deaths in liver Tx recipients [9].

Burkholderia cepacia infection in lung transplant recipients may produce a rapidly progressive pneumonia, sometimes accompanied by septicemia. Occasional patients have lung abscess or empyema [159]. Both lung transplant recipients and lung transplant candidates may have simple colonization with *Burkholderia cepacia* however. Some lung transplant candidates have a gradual but progressive decline in their clinical condition after they become colonized with *Burkholderia cepacia*. These patients may have repeated hospital admissions with fever, declining respiratory function and weight loss. In contrast, some lung transplant candidates have a rapidly progressive syndrome known as the "cepacia syndrome" [160]. These patients present with high fever and respiratory failure. Laboratory testing reveals leukocytosis and a markedly elevated erythrocyte sedimentation rate (ESR). Person to person transmission of *Burkholderia cepacia* has been reported, most likely through direct contact with respiratory secretions [161]. Transplant patients with CF and chronic granulomatous disease are vulnerable to *Burkholderia cepacia* pneumonia, and bacteremia with this pathogen may also occur.

Specific comment should be made regarding colonization and lung infection due to *Burkholderia cepacia*. Some, but not all, studies of *Burkholderia cepacia* pneumonia in lung transplant recipients have shown elevated mortality compared to patients who were never colonized with this organism. There is a report on mortality of close to 50 % in *Burkholderia cepacia* colonized CF patients undergoing lung transplantation [159]. Others found that 1-year survival of *Burkholderia cepacia* colonized patients was 67 % compared to 92 % for patients not colonized with this organism [162]. Lung SOTR with CF who were infected with *Burkholderia cepacia* had poorer outcomes and represented the majority of those who had a septic death [163]. In contrast, a number of small studies have not shown significant difference in survival of *Burkholderia cepacia* colonized versus non-colonized patients [164, 165]. It appears that a subset of *Burkholderia cepacia*, genomovar III, is linked to inferior outcome [166, 167]. Patients colonized with *B. cenocepacia* before lung transplant were six times more likely to die within one year of transplant than those infected with other *Burkholderia cepacia* complex (Bcc) species ($p = 0.04$) and eight times than noninfected patients ($p < 0.00005$) [168]. 9/12 patients with *B. cenocepacia* infection died following lung transplantation, as compared to significantly better outcomes of recipients infected with other Bcc species, comparable to other recipients with CF [169]. Therefore, colonization with *B. cenocepacia* is considered as a contraindication for lung transplantation in some centers [169].

Following lung transplantation, infection with Bcc species other than *B. cenocepacia* does not significantly impact 5-year survival whereas infection with *B. cenocepacia* pre-transplant is associated with decreased survival [168].

21.3.3 Antimicrobial Resistance

21.3.3.1 Mechanisms of Resistance

Pseudomonas aeruginosa displays a diverse array of antibiotic resistance mechanisms [170]. Resistance to beta-lactam antibiotics is usually, but not exclusively, mediated by beta-lactamases. A frightening arrival has been the IMP and VIM type beta-lactamases which can hydrolyze carbapenems, and all other beta-lactams with the exception of aztreonam [171]. However the coexistence of other beta-lactamases usually results in resistance to aztreonam. Metallo beta-lactamase production was the main mechanism of resistance in NFGNR found in one study [39].

Imipenem resistance can be mediated by loss of OprD, a porin or outer membrane protein. Loss of OprD results in resistance to imipenem and reduced susceptibility (but usually not frank resistance) to meropenem. OprD may be co-regulated with an efflux pump called MexEF-OprN [170, 172]. Use of imipenem can select for loss of OprD, but not for upregulation of the efflux pump. In contrast, use of quinolones can select for upregulation of the efflux pump and also reduced OprD (resulting in resistance to both quinolones and imipenem). Frank resistance to meropenem usually requires both loss of OprD and upregulation of an efflux pump known as MexAB-OprM [172].

The efflux pumps are an important mechanism of multidrug resistance, since they may confer resistance to quinolones, antipseudomonal penicillins, cephalosporins, and sometimes also aminoglycosides. Quinolone resistance may also be mediated by mutations to the chromosomally mediated topoisomerases II and IV. Aminoglycoside resistance may be mediated by outer membrane impermeability or by aminoglycoside modifying enzymes.

Stenotrophomonas maltophilia is intrinsically resistant to carbapenems because of the production of carbapenem hydrolyzing beta-lactamases. *S. maltophilia* usually harbors

two types of beta-lactamases: L1, a metallo-beta-lactamase that hydrolyzes all beta-lactams except aztreonam and is not inhibited by clavulanic acid and L2, an inducible class A beta-lactamase that hydrolyzes aztreonam but is inhibited by clavulanic acid. Strains harboring these beta-lactamases hydrolyze almost all beta-lactams and beta-lactam–beta-lactamase inhibitor combinations. The majority of strains are susceptible to ticarcillin–clavulanate, but not to ampicillin–sulbactam or piperacillin–tazobactam. *Stenotrophomonas maltophilia* is frequently resistant to all aminoglycosides, probably due to impermeability of the outer membrane.

A variety of beta-lactamases have been reportedly produced by *Burkholderia cepacia* [173–179]. Resistance may also be mediated by membrane impermeability.

Acinetobacter spp. may be capable of virtually complete antibiotic resistance. Some authors have used the abbreviations CRAB (carbapenem-resistant *Acinetobacter baumannii*) or PDRAB (pandrug-resistant *Acinetobacter baumannii*) [180]. As is the case with *Pseudomonas aeruginosa*, resistance of *Acinetobacter* spp. may be mediated by a combination of beta-lactamases and outer membrane protein deficiencies. A clinically useful observation has been the in-vitro efficacy of ampicillin–sulbactam in the face of resistance to almost all other drug classes. Sulbactam is able to bind to penicillin binding protein 2 (PBP-2) and therefore can impart direct antimicrobial activity against *Acinetobacter* spp [181].

21.3.3.2 Epidemiology of Resistance

Transplant recipients (HSCT and SOT) are at greater risk of MDR *Pseudomonas aeruginosa* BSI, with an appreciable mortality. In a large study, resistance to all antibiotic classes was significantly greater in *Pseudomonas* BSI isolates from transplant compared with non-transplant patients ($p<0.001$). Of isolates from transplant recipients ($n=207$), 43 % were MDR, compared with 18 % of isolates from non-transplant patients ($n=391$) ($p<0.001$) [62].

In HSCT patients *Pseudomonas aeruginosa* is frequently resistant to several antibiotic classes. 18–72 % are resistant to fluoroquinolones, 11–50 % to aminoglycosides, 15–50 % to third-generation cephalosporins, 10–28 % to piperacillin tazobactam and 8–60 % to carbapenems [15, 16, 23, 24, 72, 182]; 25–71 % are MDR [16, 62, 72, 73, 143]. Outbreaks of multidrug resistant GNR rods (*Stenotrophomonas, Pseudomonas*) were reported in HSCT units [183–185].

In SOT patients NFGNR are frequently resistant to antibiotics; 31–74 % of them are MDR in some reports [26, 29, 62, 76, 83, 186, 187]; others report on XDR *Pseudomonas* and *Acinetobacter* [76, 83]. 37 % of 49 cases of *Acinetobacter baumannii* infection in kidney and liver transplant recipients were caused by carbapenem-resistant isolates in one study [89], while in another study in liver transplant patients, 75 % were carbapenem-resistant [9]. Infection with CRAB manifested mainly as pneumonia (83 %) in one study in SOTR, half of these patients subsequently developed CRAB BSI; 5/6 patients died [188]. CRAB caused 42.9 % of all *Acinetobacter* infections in abdominal SOTR as compared to 13.7 % among non-transplant ($p<0.01$) [188]. XDR *Acinetobacter baumannii* in infections were significantly more common among cardiothoracic than abdominal transplant recipients ($p=0.0004$). Ninety-eight percent (40/41) of patients had respiratory tract infections, most commonly ventilator-associated pneumonia (VAP); 88 % [36/41]) [189].

CF patients undergoing lung transplantation are frequently infected with MDR and PDR NFGNR, such as *Pseudomonas, Burkholderia*, and others. In some centers, 44–55 % of patients harbored PDR NFGNR, mostly *Pseudomonas* [106, 190, 191]. *Burkholderia cepacia* is frequently MDR [192, 193].

Contact with other patients colonized with resistant *Pseudomonas aeruginosa* may be risk factor for acquisition of resistant strain [194].

21.3.3.3 Impact of Resistance

Mortality in MDR *Pseudomonas* infections was 36 % vs. 12.5 % in non-MDR infections in HSCT patients [182].

MDR and XDR *Acinetobacter* infections is associated with high mortality rate of 49–95 % in HSCT and SOTR [136, 188, 189, 195]. Polymyxin-resistant *Acinetobacter* colonization or infection after liver transplantation was independently associated with mortality [196].

Some studies reported on decreased survival in CF patients infected with PDR bacteria [190], others however reported that their survival is similar to patients without PDR colonization [163, 191]. Inappropriate therapy was associated with increased mortality in SOTR patients infected with *Acinetobacter* spp. and MDR GNR [39, 89].

21.3.4 Treatment

21.3.4.1 Pseudomonas aeruginosa

There has been long-standing debate over the value of combination therapy in the treatment of serious *Pseudomonas aeruginosa* infections. Combination therapy had been considered the mainstay of therapy for many years, but recently proponents of monotherapy have emerged. Much of the support for combination therapy emanated from the prospective observational study of 200 consecutive patients with *Pseudomonas aeruginosa* bacteremia, showing that combination therapy was found to be significantly better than monotherapy in improving outcome. Mortality was significantly higher in patients given monotherapy (47 %) than in patients given combination therapy (27 %) [197]. It should be noted that the most common combination used was piperacillin or ticarcillin combined with tobramycin or gentamicin. The monotherapy group was dominated by patients

given an aminoglycoside alone. Few patients received cephalosporins, aztreonam, carbapenems, or quinolones [197]. In the contrary, there are two studies, including *Pseudomonas aeruginosa*, on GNR bacteremia, that did not find statistically significant differences in mortality between those receiving beta-lactam monotherapy versus beta-lactam-aminoglycoside combination therapy [198, 199]. No difference in mortality between monotherapy with beta-lactam and combination of beta-lactam with aminoglycoside or fluoroquinolone was demonstrated in the recent review of randomized and non-randomized studies [200].

To definitively show that combination therapy is superior to monotherapy would require a randomized controlled trial of several hundred patients. It is not likely that such a study will be ever performed. The demonstration of in-vitro synergy between antipseudomonal beta-lactam antibiotics and aminoglycosides, and the development of resistance with monotherapy, prompts us to continue to recommend combination antibiotic therapy for serious *Pseudomonas* infections. It is not clear whether the combination of two antibiotics needs to be continued for the entire treatment course or whether combination therapy in the first 3–5 days of treatment is sufficient. A combination of antipseudomonal beta-lactam plus aminoglycoside is the gold standard of therapy. Minimization of the aminoglycoside component of this regimen to 3–5 days should minimize risk of toxicity [201]. Combinations of beta-lactams and quinolones are sometimes used but the clinical data to support such combinations is sparse. We do not recommend combinations of two beta-lactams. Double beta-lactam therapy has proved inferior to the beta-lactam-aminoglycoside combination in animal models [202]. One study in humans showed emergence of resistance in 40 % (two of five) of cases in one series of *Pseudomonas aeruginosa* infection treated with double beta-lactams [203].

High doses of quinolones for therapy of serious *Pseudomonas* infections are recommended. For ciprofloxacin, an intravenous dose of 400 mg every 8 h is recommended instead of standard 400 mg every 12 h. Likewise we recommend levofloxacin at 750 mg per day, rather than 500 mg per day, for serious pseudomonal infections. For beta-lactams, the rate of bactericidal activity of beta-lactams does not increase substantially once concentrations exceed four times the MIC. Beta-lactams do not exhibit a postantibiotic effect against *Pseudomonas aeruginosa* with the notable exception of the carbapenems. Thus, high drug concentrations do not kill *Pseudomonas aeruginosa* any faster than low concentrations, and bacterial regrowth will begin very soon after serum and tissue levels fall below the MIC. The duration of time that serum levels exceed the MIC is the pharmacokinetic parameter that best correlates with in vivo efficacy of the beta-lactams. Continuous infusion of antipseudomonal beta-lactams is therefore theoretically attractive. At this time, this approach remains to be validated in large clinical studies. Aminoglycosides, even when in combination therapy, should be dosed once daily. Aminoglycosides exhibit concentration-dependent bactericidal activity, and also produce prolonged postantibiotic effects. This supports the practice of once daily aminoglycoside dosing.

21.3.4.2 Other NFGNR

There are no randomized controlled trials which can guide therapy of *Stenotrophomonas maltophilia*. Trimethoprim–sulfamethoxazole should be considered the primary therapeutic agent. Resistance may arise and the sulfonamide component is poorly tolerated by some patients [204–206]. However, it must be recognized that *Stenotrophomonas maltophilia* may be a colonizer of the airways, in which case not treatment is needed. Alternative agents against *Stenotrophomonas maltophilia* proposed by some authors include the beta-lactams, ticarcillin–clavulanate, and ceftazidime; fluoroquinolones, with moxifloxacin reportedly active in-vitro against some ciprofloxacin-resistant isolates from hematological patients [207]; minocycline and chloramphenicol. Combination therapy with either ticarcillin–clavulanate or with a third-generation cephalosporin (mainly ceftazidime) should be considered in a neutropenic or severely ill patients [204, 208, 209]. Published cases series on treatment regimens other than trimethoprim–sulfamethoxazole are small with variable success and drugs used often in combination [50, 204–206].

Burkholderia cepacia can be extremely resistant, but ceftazidime, carbapenems (imipenem and meropenem), ciprofloxacin, piperacillin, chloramphenicol, and trimethoprim–sulfamethoxazole have the greatest likelihood of in vitro activity. It is important to note that combination therapy is highly desirable because of the probability of emergence of more resistant isolates during therapy. *Burkholderia cepacia* is resistant to commonly used inhaled antibiotics (tobramycin and colistin) [106].

Carbapenems (for example, imipenem or meropenem) have traditionally been regarded as extremely potent agents in the treatment of severe infections due to *Acinetobacter* spp. This has been borne out in studies of *Acinetobacter* bacteremia [210]. Carbapenem-resistant *Acinetobacter baumannii* may remain susceptible to sulbactam [2, 168, 211] a beta-lactamase inhibitor that also has clinically relevant intrinsic antimicrobial activity against the organism. In patients with strains resistant to virtually all currently available antibiotics, colistin may be the only viable option [212]. In-vitro studies show potential advantages of combinations of rifampin with colistin [213]. A new antibiotic, tigecycline, shows usefulness against multiresistant *Acinetobacter* organisms [65, 214]. *A. baumannii* can develop resistance to tigecycline by mutation, with the trait sometimes selected in therapy [215–217]; moreover some regionally prevalent MDR strains are non-susceptible to tigecycline [218].

21.4 Treatment Options for Carbapenem-Resistant GNR

Treatment of carbapenem-resistant GNR is challenging. In some cases, the only treatment options include old antibiotics (polymyxins and fosfomycin), tigecycline, and aminoglycosides [50, 135, 219–221]. All these options have efficacy, resistance, and/or toxicity issues.

Summary on current treatment options for carbapenem-resistant GNR is presented in Table 21-1.

21.4.1 Polymyxins

The polymyxins were originally isolated from *Bacillus* spp—polymyxin B from *B. polymyxa* in 1947 and colistin (also known as polymyxin E) from *B. colistinus* in 1950. The polymyxins act primarily on the bacterial cell wall, leading to rapid permeability changes in the cytoplasmic membrane. Entry into the cell is not necessary. The polymyxins may also have antiendotoxin activity. Carbapenem-resistant GN can remain sensitive to colistin. Increasing number of reports on successful systemic polymyxins use, including in transplant patients [222–224]. Other usages of colistin reported were: as aerosols, in adjunction to systemic therapy in patients with pneumonia [225], intraventricular use for CNS infections [226] and endotoxin removal using polymyxin-B-based hemoperfusion [227]. Inhaled colistin in lung transplant patients may delay colonization with *Pseudomonas aeruginosa* [228]. The use of colistin raises several issues of concern:

1. Efficacy. Treatment with colistin was associated with increased mortality as compared with other appropriate regimens in several studies; some of them included onco-hematological patients [229, 230]. Others, however, reported on considerable effectiveness, depending on the daily dosage and infection site [222, 223, 231].
2. Toxicity, mainly nephrological and neurological. Nephrotoxicity, which was reported in up to 50 % of patients receiving colistin–polymyxin B in older studies,

Table 21-1. Main characteristics of the new or revisited antibacterial drugs for treatment of infections due to MDR GNR bacteria

	Colistin/polymyxin B [115, 220, 221, 232, 235, 236, 305]	Tigecycline [216, 220, 244, 306]	Fosfomycin [204, 231, 307, 308]
Class	Polymyxin	Tetracyclines	Phosphonic acid derivative
Mechanism of action, hydro/lipophilic	Disrupts bacterial membranes, hydrophilic	Protein synthesis inhibition, lipophilic	Inhibits peptidoglycan synthesis, Hydrophilic
Bactericidal/-static; concentration/time dependent activity	Bactericidal, concentration dependent	Bacteriostatic, time dependent	Bactericidal, variable concentration-dependent or time-dependent
Spectrum	Enterobacteriaceae, *P. aeruginosa*, *A. baumannii*, *S. maltophilia*, not *Proteus, Serratia, Providencia* spp	Enterobacteriaceae, *A. baumannii, S. maltophilia,* not *P. aeruginosa, Proteus, Morganella*, and *Providencia* spp	Enterobacteriaceae (esp. *E. coli*), some *P. aeruginosa,* not *A. baumannii*
Half life	5.9 ± 2.6 h (Following administration of two million international units of colistin methanesulphonate)	37 ± 12 h	5.7 ± 2.8 h
Route of elimination	Renal	Biliary/fecal and renal	Renal and fecal
Dose and route	Wide dose range used (3–9 × 10^6 IU/day) Loading dose nine million IU and maintenance dose 4.5 million IU every 12 h preferred, IV	100 mg loading dose followed by 50 mg twice daily, IV	Range 2 g three times daily up to 4 g four times daily, IV
Main side effects	Nephrotoxicity, neurotoxicity	Nausea, vomiting and headache	Gastrointestinal (rare)
Warnings	Increased mortality as compared to other appropriate regimens in some retrospective studies Low colistin concentration after the first few doses in the routine dose regimen	Low blood levels Increased risk of death compared to other antibiotics used to treat severe infections	No clinical experience in this patient population Readily selects resistance
European Medicines Agency (EMA) labeled indications	Serious infections caused by GNR bacteria, including those of the lower respiratory tract and urinary tract where sensitivity testing suggests that they are caused by susceptible bacteria	Complicated skin and soft tissue infections, complicated intra-abdominal infections	No EMA license; individual country licenses include infections of lung, urinary tract, and bone, with associated bacteremia

Adapted from Averbuch D, Cordonnier C, Livermore DM, Mikulska M, Orasch C, Viscoli C et al. Targeted therapy against multi-resistant bacteria in leukemic and hematopoietic stem cell transplant recipients: guidelines of the 4th European Conference on Infections in Leukemia (ECIL-4, 2011). *Haematologica* 2013; 98(12): 1836–47.

is much less frequent in newer studies, including HSCT patients, with rates ranging from 10 to 30 % [221–224, 232].

3. Appropriate dose. The recommended dose in adults is nine million IU daily in two or three divided doses as a slow intravenous infusion; in critically ill patients a loading dose of nine million IU should be given. Doses should be reduced according to creatinine clearance in patients with renal impairment. In children, the suggested dose is 75,000 to 150,000 IU/kg daily, in three divided doses [233]. Loading dose and high daily dosages of colistin may help to overcome the problem of low blood levels that may have been responsible for the suboptimal efficacy of polymyxins, as well as to the selection of resistant strain variants [219, 234–237].
4. Emergence of colistin-resistant GN after previous exposure to colistin was reported [196, 234]. Susceptibility decreased during therapy with colistin in 40 % of SOTR infected with XDR *Acinetobacter baumannii* [195].

21.4.2 Tigecycline

Tigecycline has a broad spectrum of in vitro activity against MDR GNR bacteria, excluding *Pseudomonas aeruginosa, Proteus* spp., *Providencia* spp., *and Morganella* spp. [205, 238–240]. Standard dosage tigecycline, in combination with an anti-pseudomonal drug (ß-lactams, quinolones, aminoglycosides) achieved clinical response in 56 % of HSCT recipients [241]. Patients with pneumonia had lower response and higher mortality rates than those with bacteremia (51 % vs. 79 %, 44 % vs. 16 % respectively, both $p < 0.05$) [241]. In another study, standard dosage tigecycline used alone or combined with other antibiotics, showed clinical response (defined as partial or complete improvement of signs/symptoms of infection) in 16/23 (70 %) of bacteremia cases, 18/29 (67 %) of pneumonia and in 7/12 (58 %) where it was used for empirical treatment of febrile neutropenia [242],. The microbiologic response rate 70 % was achieved during treatment of CRKp infections after liver transplantation in the intensive care unit (ICU) with tigecycline, but 30 % died due to CRKp [243].

Higher-dosage tigecycline regimens potentially may be advantageous in severe infections. A recent randomized study in patients with hospital-acquired pneumonia showed that clinical cure with tigecycline 100 mg twice daily after a loading dose of 200 mg (17/20, 85.0 %) was numerically higher than with tigecycline 75 mg twice daily after a loading dose of 150 mg (16/23, 69.6 %) and imipenem–cilastatin (18/24, 75.0 %) [154]. However, evidence of increased mortality, compared to other antibiotic therapies, especially in VAP [244] leads to caution in its use. Moreover, a serious drawback, at least for monotherapy in bacteremia, is the low serum level obtained [216]. Superinfections with pathogens inherently resistant to tigecycline (*Pseudomonas aeruginosa*, *Proteus* spp., *Providencia* spp., and *Morganella* spp.) are another concern [215, 236, 243]. Breakthrough CRE bacteremia during tigecycline therapy was reported due to susceptible strains [113]. Increased MIC during treatment with tigecycline was reported in kidney Tx patient [245].

21.4.3 Fosfomycin

Fosfomycin is another old, but increasingly revisited, antibiotic with broad-spectrum in vitro activity against GNR bacteria, excluding *Acinetobacter* spp. Several studies estimate 80–90 % of Enterobacteriaceae with extended-spectrum beta-lactamases (ESBLs) and carbapenemases to be susceptible to fosfomycin [204, 219], but other studies report that only 50 % of *Klebsiella* spp. and fewer than 30 % of MDR *Pseudomonas aeruginosa* to be susceptible [135, 221]. Due to the possibility of resistance developing during therapy, fosfomycin should be used in combination with other agents, selected according to the susceptibility results [204, 246]. Data on the efficacy of intravenous fosfomycin are limited to case reports and small case series [204] and there is no published experience of treating invasive infections in oncohematological and HSCT patients. A retrospective study in HSCT patients showed, that in a multivariate analysis, exposure to fosfomycin (route of administration not specified) was associated with a significantly decreased incidence of veno-occlusive liver disease [247]. Fourteen cases of UTI in kidney Tx recipients were treated with fosfomycin, mostly due to *E.coli*, 50 % resistant to carbapenems. The overall clearance rate of UTI at 3 months was 31 %; recurrence occurred in 54 % and persistence occurred in 21 % of cases, no adverse drug reactions were reported [248]. In another report, 30 % microbiological cure was achieved when MDR GNR UTI was treated with fosfomycin in 15 SOTR; in 3 of them resistance to fosfomycin developed during treatment, and another one had superinfection due to fosfomycin-resistant bacteria [249].

21.4.4 Combination Therapy in Infections Due to Resistant GNR

21.4.4.1 In-Vitro Data

Some in vitro data suggest synergy in combining two agents (polymyxin B and either rifampin or doxycycline; fosfomycin with meropenem or colistin) against carbapenemase-producing *Klebsiella pneumoniae*, even when the pathogen is resistant to one of these agents [250, 251]. An ertapenem–doripenem combination may be of potential usefulness against KPC-producing *Klebsiella pneumoniae* based on a study in an immunocompetent murine thigh infection model based on the notion that the high affinity of KPC for ertapenem would “trap” the enzyme thus enhancing the activity of doripenem [252].

A recent meta-analysis of studies examining in vitro interactions of antibiotic combinations consisting of any carbapenem with colistin or polymyxin B against GNR reported that combination therapy showed synergy rates of 77 % for *Acinetobacter baumannii*, 44 % for *Klebsiella pneumoniae*, and 50 % for *Pseudomonas aeruginosa*, with low antagonism rates for all. Doripenem showed high synergy rates for all three bacteria. The use of combination therapy led to less resistance development in vitro [253].

Various combinations of rifampin, beta-lactams, aminoglycosides, quinolones, colistin–polymyxin B, fosfomycin, or other agents are synergistic in vitro, or in animal models, against MDR *Pseudomonas* or *Acinetobacter* spp. [135, 211, 254–258].

21.4.4.2 Clinical Data

In the era of increasing resistance, combination therapy is increasingly used for treatment of carbapenem-resistant and MDR GNR [221].

Several meta-analyses of randomized controlled studies, some of them done before the present era of increasing resistance, concluded that there was similar all-cause mortality in febrile neutropenic patients treated with a beta-lactam vs. the same beta-lactam plus an aminoglycoside as empirical or definitive therapy [148, 259, 260]. However, owing to the small numbers of cases of infection due to resistant bacteria, a benefit of combination therapy could not be ruled out for those patients who were critically ill or were infected with *Pseudomonas aeruginosa* or some other resistant pathogen [148, 259, 260].

In a recently published prospective multicenter study which compared empirical therapy with piperacillin–tazobactam with or without tigecycline in high-risk neutropenic patients with hematologic malignancies, the combination therapy proved to be more effective, including in patients with bacteremia and clinically documented infections [261].

A retrospective study reviewed patients with hematological malignancies or post-HSCT, who were infected by ESBL- or AmpC-producing Enterobacteriaceae or resistant *Pseudomonas*, most of whom were empirically treated with combination of a beta-lactam and an aminoglycoside. Mortality was lower among those patients whose pathogen was sensitive in vitro to either the beta-lactam or the aminoglycoside, compared with those whose pathogen was resistant to both (OR, 1.8; 95 % CI, 1.3 to 2.5) [87].

Carbapenem-containing combinations were associated with significantly reduced mortality compared to non-carbapenem-containing regimens in a retrospective analysis of 138 patients who received treatment for infections due to carbapenemase producing *Klebsiella pneumoniae* when the carbapenem MIC for the infecting organism was ≤4 mg/L [122]. Patients infected with CRKp who received combination therapy, especially with a combination of tigecycline, colistin, and meropenem, had lower mortality as compared to monotherapy treated group [262]. Combination antibiotic therapy improves the likelihood that at least one component agent is active in patients with severe sepsis or septic shock associated with GNR bacteremia [263].

The combination of a carbapenem and colistin was successfully used in SOT patients infected with CRKp and XDR *Acinetobacter baumannii* [116, 189, 195]. This combination was associated with improved survival in XDR *Acinetobacter baumannii* infections and decreased chance of development resistance to colistin as compared to other combinations [189]. In a recently published case-control study in critical patients in ICU infected with carbapenem resistant GNR, mainly *Pseudomonas aeruginosa*, combination therapy had been used significantly more often in survivors compared with non-survivors (32.1 % vs. 7.8 %, $p < 0.01$) [264].

Rifampin was considered for addition to other active antibiotics in the treatment of uncontrolled infection due to MDR bacteria [211, 240, 250, 265–267]. However, a randomized, open-label clinical trial, which enrolled 210 patients with life-threatening infections due *Acinetobacter baumannii* that were susceptible only to colistin showed that 30-day mortality was not reduced by addition of rifampicin [268]. Similarly, another randomized controlled study comparing colistin to combination of colistin and rifampin for VAP caused by *Acinetobacter* did not show significant differences in mortality [269]. Other problems with rifampin include its toxic potential and drug interactions, a main concern especially in transplant patients who receive a lot of other drugs concomitantly (such as cyclosporine, mycophenolate mofetil, antifungals, antivirals) [268].

Although several studies reported on the improved outcome in patients who received combination therapy, mainly including colistin, a summary of the studies (12 retrospective cohort studies or case series, two prospective observational studies and two randomized controlled studies) did not demonstrate difference in mortality between colistin alone and colistin–carbapenem combination therapy for the treatment of carbapenemase-producing GNB or carbapenem-resistant GNR [270].

Two randomized controlled studies are currently recruiting patients, comparing colistin–carbapenem combination therapy versus colistin monotherapy for invasive infections caused by MDR and XDR-GNB, will clarify this issue (NCT01732250, NCT01597973).

Aerosolized colistin can be considered as an adjunctive therapy for MDR infections causing pneumonia. A successful use of 100–150 mg colistin, administered via a Respirgard II nebulizer, as part of combination therapy for nosocomial pneumonia caused by MDR *Pseudomonas aeruginosa* was described [271]. Potential concerns over aerosolized colistin include development of resistance to the antibiotic [272]. In a retrospective study which compared treatment of colistin-only susceptible GNR bacteria with intravenous (IV) colistin vs. aerosolized colistin in adjunction to IV colistin, patients who received the adjunction therapy had a higher clinical cure rate required fewer days of mechanical ventilation after VAP onset [225].

21.5 Prevention

A meta-analysis of 109 trials performed during 1973–2010 reported that antibiotic prophylaxis, especially with quinolones, in afebrile neutropenic patients significantly reduced all-cause mortality [273]. Antibacterial prophylaxis with a fluoroquinolone (levofloxacin or ciprofloxacin) to prevent bacterial infections was recommended for adult SCT patients with anticipated neutropenic periods of 7 days or more. Antibacterial prophylaxis is generally started at the time of stem cell infusion and continued until recovery from neutropenia or initiation of empirical antibacterial therapy for fever during neutropenia. The prophylaxis should not be continued after recovery from neutropenia. Quinolone prophylaxis, however, has to be reconsidered in the situation of growing resistance. Local epidemiological data should be carefully considered before applying fluoroquinolone prophylaxis and once it is applied, the emergence of resistance in bacterial pathogens should be monitored closely because of increasing quinolone resistance worldwide [54]. Prophylaxis efficacy may be reduced when the prevalence of fluoroquinolone GNR bacillary resistance exceeds 20 % [274, 275]. Although a meta-analysis of 27 studies, published at 2007, reported on nonsignificant increase in colonization by quinolone-resistant bacteria under quinolone prophylaxis [276], later studies reported that infections which emerge under quinolone prophylaxis can be caused by MDR bacteria, necessitating use of broader spectrum antibiotics for treatment [277, 278]. Possible benefit of quinolone prophylaxis has to be considered based on local epidemiology and resistant data and if prophylaxis is discontinued—outcome of bacterial infections has to be closely monitored.

21.5.1 Prevention of Resistance

Efforts to reduce antibiotic resistance among transplant patients must address two directions: limitation of use of broad spectrum antibiotics and disruption of spread of resistant bacteria.

Heavy antibiotic use has been constantly reported as one of the main factors for development of resistant bacteria. Limitation of unnecessary use of broad spectrum antibiotics is important to reduce the spread of resistance.

The ECIL group has proposed guidelines for empirical antibiotic therapy in the era of growing resistance [279]. Initial antibiotic regimen has to be targeted on the most prevalent bacteria at the center, unless the patient is seriously ill at presentation or is known to be colonized or previously infected with resistant bacteria. Differential approaches should be implemented for febrile neutropenic patients based on their presentation, knowledge on colonization/previous infection with resistant bacteria and local epidemiology in each center. An escalation strategy is recommended for patients with uncomplicated presentation, who are unknown to be colonized or previously infected with resistant bacteria, in centers where infections due to resistant pathogens are rarely seen at the onset of febrile neutropenia. Such patients should be treated empirically with either anti-pseudomonal cephalosporins (cefepime, ceftazidime), or beta lactam-beta lactamase inhibitors (piperacillin–tazobactam, ticarcillin–clavulanate, cefoperazone–sulbactam) or combination of piperacillin and gentamicin. Usage of carbapenems and combinations should be avoided in such patients. Modifications of the initial regimen at 72–96 h should be based on the patient's clinical course and the microbiological results. The ECIL guidelines defined situations in which use of carbapenems and combination therapy is justified (de-escalation approach), specifically in seriously ill patients, e.g., presentation with septic shock; those known to be colonized or previously infected with resistant bacteria or in centers with a high prevalence of infections due to resistant bacteria at the onset of febrile neutropenia. This de-escalation approach has to be followed by discontinuation of combination therapy or switch to a narrower-spectrum regimen in patients who were stable since presentation and in whom resistant bacteria was not isolated, especially if fever normalized.

The empirical antibacterial treatment can be discontinued at ≥72 h irrespective of neutrophil count or expected duration of neutropenia in patients without evidence of clinically or microbiologically documented infections, who are hemodynamically stable since presentation and afebrile ≥48 h [280]. The patient should be kept hospitalized for at least 24–48 h under close observation if he is still neutropenic when antibiotic therapy is stopped. If fever recurs, antibiotics should be restarted urgently. This strategy aims to limit exposure to broad spectrum antibiotics and combinations, and also duration of antibiotic treatment, minimizing the collateral damage associated with antibiotic overuse, and the further selection of resistance.

Promising new diagnostic techniques enabling rapid (within few hours) identification of ESBL and carbapenemase-producing bacteria, with high sensitivity and specificity, may contribute to avoid of overuse of carbapenems [281–283]. The problem is that these tests should be applied on positive blood cultures, meaning that still ~24 h (ideally) will pass from the onset of infection until the result of these tests will be available. These tests can miss some carbapenemases in some bacteria (e.g., OXA-48, *Pseudomonas aeruginosa*) and they do not detect carbapenem-resistant bacteria due to mechanisms other than carbapenemases (e.g., reduced permeability of the outer membrane associated with overexpression of chromosomal or acquired AmpC and/or ESBL [284–286].

Antibiotic stewardship is crucial to use antimicrobials in such a way that each and every patient receives the most efficacious and safe antimicrobials to treat their infections, while at the same time minimizing the ecologic impact of

antimicrobials used [287]. Five main principles of antibiotic stewardship in HSCT patients were defined [288]:

1. Local surveillance of antibiotic resistance, antibiotic consumption and patient outcomes, including monitoring reports;
2. Multidisciplinary protocols and algorithms on the diagnosis, prevention and treatment of infections should be developed in collaboration of oncologists, infectious disease specialists, and medical microbiologists and updated to reflect changes in bacterial antimicrobial susceptibilities in the unit;
3. Swift reporting of positive clinical cultures and implementation of rapid techniques for bacterial identification and resistance patterns by the microbiology laboratory to control the duration of treatment and to facilitate reassessment of the antibiotic therapy;
4. Optimization of dosing regimens using pharmacodynamic principles;
5. Frequent multidisciplinary rounds including discussion of patient histories and interactive, bedside education on antimicrobial drug use and infection control.

Infectious control is crucial to prevent spread of resistant bacteria between patients within department, as well as between departments in the hospital and between hospitals. Antimicrobial resistance is a worldwide problem. Transportation of patients between departments in the same hospital, as ICU, surgery and transplant ward, between different hospitals, as well as medical tourism, contributes to the spread of resistant bacteria across the borders. Horizontal transmission of ESBL-producing *Klebsiella*, from patient to patient, via the hands of staff members has been very well documented [289–291]. Interventions to prevent and control the spread of MDR bacteria include hand-hygiene measures; active screening of patients with cultures; contact barrier precautions; enforcement of isolation criteria for patients colonized or infected with multidrug-resistant organisms; the use of single rooms for HSCT recipients; cohorting of infected patients; environmental cleaning and anti-infective stewardship [288, 292, 293]. Bundles including combination of multiple interventions were efficient for containment of carbapenem-resistant Enterobacteriaceae [99, 291]. Avoidance of contact with resistant *Pseudomonas* infected patients is important to prevent MDR *Pseudomonas* acquisition in lung transplant recipients [194].

Rapid detection and isolation of patients colonized with resistant bacteria can limit its spread. Novel molecular-based diagnostic screening tests enable simultaneous detection of several resistant bacteria directly from swab samples with high sensitivity, specificity, positive predictive value, and negative predictive value and results available in 24 h [294, 295].

Decolonization of patients colonized with CRE with oral aminoglycoside or colistin was successful in 37–68 % of patients, although the appropriate dose has to be determined and there is concern that those who remained colonized will be colonized with resistant bacteria after de-colonization [296–301].

Transmission of microorganisms from an infected brain-dead donor can cause severe, sometimes fatal infection in the SOT recipient, even if appropriate therapy is provided [115, 302, 303]. On the other hand, the donor pool is limited and increasing numbers of donors have underlying diseases, and may be infected with MDR bacteria. Investigation of donors for CRE carriage by suitable approaches (e.g., rectal swabbing) would seem mandatory, especially in areas where CRE are endemic [302]. A systematic approach for the acceptability of organs from donors infected with MDR bacteria was suggested, based on expert opinion [304]. The algorithm includes screening for MDR GNR in potential donors, who are at risk for MDR infection. If a donor was found to be colonized/infected with MDR bacteria, prophylactic antibiotic treatment should be initiated to donor and to recipient, with the appropriate agent according to susceptibility testing. Two conditions are contraindication to SOT: (1) if the donor has MDR bacteremia (2) lung transplantation from donor infected/colonized with MDR bacteria for which no adequate antibiotic treatment for pneumonia exists [304].

21.6 Summary

Infections caused by GNR are increasingly common in transplant recipients; they can cause severe, life-threatening diseases. Prevention approaches, early diagnosis, appropriate empiric therapy based on local epidemiology and proper targeted therapy are crucial for patients survival. There is a global problem of growing resistance among GNR and it compromises prophylaxis and treatment options. Previous colonization and exposure to antibiotics are the most important risk factors for the development of resistance. Treatment of carbapenem-resistant GNR is challenging; in some cases, the only treatment options include old antibiotics (polymyxins and fosfomycin), tigecycline, and aminoglycosides. All these options have efficacy, resistance, and/or toxicity issues. Development of new treatment modalities is an important goal. Continuous monitoring of the local epidemiology and antimicrobial stewardship is mandatory for optimization therapy with the currently available drugs. Infectious control is crucial to limit the spread of resistance.

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22
Typical and Atypical Mycobacterium Infections After Hematopoietic Stem Cell or Solid Organ Transplantation

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22.1 Bacteriology

Mycobacteria share the staining characteristic referred to as *acid fastness* [1]. These include the organisms causing tuberculosis (*Mycobacterium tuberculosis* and *Mycobacterium bovis*) and a variety of other mycobacterial species of varying pathogenicity and clinical importance. The term *acid-fast bacilli* is, practically speaking, the identifying feature of mycobacteria, although some other non-mycobacterial microbes, notably *Nocardia*, are variably acid fast.

From a clinical standpoint, mycobacteria can be divided into the following four broad classes: *Mycobacterium tuberculosis* complex (*M. tuberculosis*, *M. bovis*, *M. bovis-BCG*, *M. africanum*, *M. microti*, and *M. canetti*), *Mycobacterium avium* complex (*M. avium* and *M. intracellulare*), Hansen's disease (leprosy, *M. leprae*), and the rest. The last class is also referred to as nontuberculous mycobacteria (NTM), atypical mycobacteria, or anonymous mycobacteria [2]. The organisms of *M. tuberculosis* complex can be differentiated from other mycobacteria by their in vitro culture characteristics, including slower growth (typically >10 days); lack of pigment; niacin production; and, most often, sensitivity to isoniazid (INH). The atypical mycobacteria grow more rapidly, usually lack niacin production, produce heat-resistant catalase in large amounts and are highly resistant to INH. In stained preparations, *M. tuberculosis* demonstrates serpentine cord formation, whereas nontuberculous atypical mycobacteria orient randomly. Using the culture characteristics of NTM, Timpe and Runyon [1, 3] proposed a useful method of classification based on colony characteristics, the rate of growth in culture, and pigment production. The four major groups are referred to as photochromogens, scotochromogens, nonchromogens, and rapidly growing mycobacteria. More sophisticated methods have been used to speciate mycobacteria in culture. These include DNA hybridization methods that use highly specific DNA probes, polymerase chain reaction (PCR)-based sequencing, computer-assisted gas liquid chromatography, or matrix assisted laser desorption-time of flight mass spectrometry (MALDI-TOF MS) [4–7]. These techniques can readily speciate mycobacteria, but they are time and labor intensive, they require pure cultures, and they generally have been available only in specialized laboratories. However, their clinical application, especially MALDI-TOF MS, is becoming more widespread, thus allowing the rapid early identification of mycobacterial species growing in vitro.

22.2 Epidemiology and Pathogenesis

M. tuberculosis is an aerobic, nonspore-forming, nonmotile, and slow-growing bacillus with a lipid-rich cell wall. Humans are the only known reservoir of this organism. Almost all infections are caused by the inhalation of infectious particles aerosolized by coughing, sneezing or talking. *M. tuberculosis* can survive only for extremely short periods outside the human body. Fomites, therefore, are rarely responsible for transmitting infection. *M. tuberculosis* rarely, if ever, has a commensal presence. The isolation of *M. tuberculosis* from pathologic specimens should therefore always be considered evidence of infection.

Atypical mycobacteria are free-living organisms that are ubiquitous in nature. They have been found in soil, water, domestic and wild animals, milk, and fruit products. They have been known to colonize body surfaces and secretions and they frequently can contaminate clinical specimens from the environment [8–11]. Therefore, unlike *M. tuberculosis*, in which the isolation of even a single colony is considered evidence of infection and is always viewed as clinically significant, atypical mycobacterial species may colonize body surfaces and secretions for prolonged periods without causing disease. Differentiation between contamination, colonization, and disease is often difficult. Guidelines have been proposed to facilitate making such decisions [12, 13]. Characteristics of a clinically significant atypical mycobacterial infection include a clinical presentation compatible with atypical mycobacterial infection; the culture of the pathogen from a normally sterile site, such as cerebrospinal or pleural fluid or a liver biopsy specimen; and/or repeated isolation of potential mycobacterial pathogens from nonsterile

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sites. The isolation of a species not known to cause human disease should be considered of clinical importance, because the clinical site sampled had some signs or symptoms of infection, particularly in an immunocompromised host.

Atypical mycobacterial infections may occur more often in the compromised host because immune deficiency contributes to the pathogenesis of mycobacterial infections. In the presence of normal host immune function, the organization of lymphocytes, macrophages, and Langerhans giant cells results in granuloma formation and the containment of infection [14]. When host immunity is compromised, the tissue reaction can be minimal or nonexistent, and uncontrolled proliferation of mycobacteria can continue without granuloma formation [15] or any effective means to control infection. Patients undergoing blood and bone marrow or solid organ transplantation require potent, and often prolonged, immunosuppressive therapy. These patients, therefore, have an increased risk of a variety of infections, including mycobacteria [16]. Furthermore, because the host response to these infections is limited, these patients may often present with atypical features, making the diagnosis difficult and the response to therapy suboptimal [16].

22.3 Immune Defects in Transplant Recipients That Are Permissive for Mycobacterial Infection

The incidence of mycobacterial infections is higher in transplant recipients than that observed in the general population. However, the rate in hematopoietic stem cell transplantation (HCT) recipients is not as high as that seen in solid organ transplant recipients. This may be due, at least in part, to the prolonged and often lifetime duration of immunosuppression in the solid organ recipients compared with that for the usual HCT recipient. Marrow allograft recipients typically recover adequate immune function in 9–12 months, unlike organ transplant recipients who experience immunosuppression for several years or throughout their lives. Therefore, the transient immune compromise experienced by HCT recipients may induce only a limited risk period of infection compared with that of organ allograft recipients.

Therapeutic immunosuppression to prevent graft rejection creates a permissive milieu for mycobacterial replication. The ideal immunosuppressive agent, which would prevent graft rejection while preserving antimicrobial immunity, is unavailable. Although agents differ in their mechanism of action and the aspects of immune function that they affect, all agents have increased infection risk as one of their major side effects.

Corticosteroids are commonly included in most immunosuppressive regimens to prevent graft rejection. The spectrum of host defense defects induced by corticosteroids includes the suppression of macrophage function; the blunting of acute inflammation; the inhibition of the T-cell activation cascade yielding impaired cellular immunity; and impaired antibody production [17–19]. Corticosteroid effects appear to be dose dependent, although a threshold dose below which immune function is unaffected is not apparent [20, 21].

Azathioprine, a purine analogue, has been a common component of immunosuppressive regimens for preventing organ allograft rejection, although less often used in recent years. After its in vivo conversion to 6-mercaptopurine, it inhibits purine nucleotide synthesis, thereby preventing antigen-induced lymphocyte proliferation and leading to impairments in natural killer cell activity, generation of cytotoxic T-cells and antibody production by B-cells [22]. In addition, azathioprine is myelosuppressive, and the resulting leukopenia further adds to infection risk.

Cyclosporine A, which was introduced in the early 1980s, has had a major impact on the prevention of graft rejection and improvement in graft survival rates. Unlike corticosteroids and cytotoxic agents, cyclosporine has a narrowly focused effect on T helper cells, while sparing cytotoxic T-cell function [23]. Its major action is to block the antigen-induced T-cell expression of lymphokines, including interleukin (IL)-2, IL-3, IL-4, interferon gamma, and tumor necrosis factor-α [24, 25]. Natural killer cells and macrophages are probably unaffected by cyclosporine. Unlike azathioprine and other cytotoxic agents, cyclosporine does not result in bone marrow suppression.

Patients on immunosuppressive regimens containing cyclosporine are at a lower risk of infection than those on non-cyclosporine immunosuppressive regimens [26, 27]. The risk of mycobacterial infections in cyclosporine-treated patients also appears to be less than in noncyclosporine-treated patients [28], although randomized comparative studies reporting the risks of mycobacterial infections are not available. Tacrolimus is nearly ten times more potent than is cyclosporine, and it has a similar, but distinct mechanism of action. The risk of infection in tacrolimus-treated patients is expected to be similar to that observed in cyclosporine-treated patients.

Other agents have been broadly applied for prevention of rejection in solid organ transplantation and graft-versus-host disease (GVHD) prophylaxis after HCT. Mycophenolate mofetil is a prodrug of mycophenolic acid with potent immunosuppressive capacity. It selectively and reversibly inhibits inosine monophosphate dehydrogenase and thereby blocks the de novo pathway of purine synthesis in lymphocytes. It has recognized additive and synergistic immunosuppressive activity with calcineurin inhibitors (cyclosporine and tacrolimus). Due to its lesser myelosuppressive activity it has frequently replaced methotrexate in GVHD prophylaxis for reduced intensity conditioning HCT and is widely used following umbilical cord blood grafting. Its broad T-cell immunosuppressive effects augment risks of infection, but no specific relationship to mycobacterial infection has been recognized.

Sirolimus is now broadly applied to prevent kidney and liver rejection and has promising activity in GVHD prophylaxis. It is a naturally occurring compound from a soil fungus and in addition to its immunosuppressive properties, sirolimus has antifungal, antiviral and antineoplastic properties, although limited in potency. Similar in mechanism of action to calcineurin inhibitors, sirolimus additionally binds to FK-binding protein 12 and then complexes with mammalian target of rapamycin (MTOR) to blunt T cell activation and proliferation. It inhibits IL-2 driven signaling from the T cell receptor and has activity limiting dendritic cell activation through reduced antigen uptake, cellular maturation, intracellular signaling, and apoptosis. Its side effects include modest impairment of renal function, but it induces hyperlipidemia and in combination with calcineurin inhibitors, increases risks of thrombotic microangiopathy. Although its broadly immunosuppressive capacity could augment infection, no specific reports of its relation to mycobacterial infection are available.

Antilymphocyte globulin, antithymocyte globulin, alemtuzumab, and monoclonal anti-T-cell antibodies (OKT3) have long been used for the treatment of graft rejection. Reports indicate a significant increase in the incidence of bacterial and viral infections with the use of these agents. One report suggests that OKT3 use led to a mycobacterial infection [29]. However, specific data on the effect of these agents on mycobacterial infections are not available [30–32].

22.4 Screening and Secondary Chemoprophylaxis

Screening for latent tuberculosis infection is recommended prior to organ transplantation [33, 34]. Transplant candidates at risk for reactivation of latent tuberculosis can be readily identified during the pretransplantation evaluation based on residence in endemic areas or a history of exposure to tuberculosis. These transplant candidates should undergo tuberculin skin testing (TST) or testing with an ex vivo interferon-gamma release assay (IFGRA) such as T-spot or QuantiFERON-TB Gold [35–38]. TST alone is an imperfect identifier of high-risk patients, because as many as 80 % of transplant candidates may be skin test anergic [39, 40]. For example, the QuantiFERON-TB Gold IFGRA test measures the release of interferon-gamma in fresh heparinized whole blood in response to stimulation of synthetic peptides representing two proteins present in *M. tuberculosis*: early secretory antigenic target-6 and culture filtrate protein-10 [41, 42]. It distinguishes between active pulmonary tuberculosis and nontuberculous mycobacterial infections [43].

In the general population, QuantiFERON-TB Gold has 96–99 % specificity that is unaffected by prior bacille Calmette-Guérin vaccination [44, 45]. In a study of 153 patients with chronic liver disease awaiting transplantation, 37 (24 %) had a positive TST and 34 (22 %) had a positive QuantiFERON-TB Gold assay, with overall agreement between the tests of 85 % [46]. Discordant test results were noted in both directions: 12 TST positive patients had negative QuantiFERON-TB Gold assays, whereas nine patients with positive QuantiFERON-TB Gold assays had negative TST. Indeterminate QuantiFERON-TB Gold assays were found among patients with low lymphocyte counts and high model for end-stage liver disease (MELD) scores. There is a need for caution when interpreting a negative QuantiFERON-TB Gold test in an endemic country, as illustrated by a case of tuberculosis that developed in a liver transplant recipient in Italy [47].

Positive QuantiFERON-TB Gold testing, in the setting of negative TST, frequently anticipates the booster phenomenon, helping to explain the discordance between the two tests [48]. In the setting of a pretransplant evaluation, which may occur over several days and is not amenable to serial TST testing, performing both TST and QuantiFERON-TB Gold testing may be helpful in finding most cases of latent tuberculosis infection.

Previous bacille Calmette-Guérin immunotherapy has been reported to result in reactivation after transplantation [49–51]. INH chemoprophylaxis has been suggested for patients with previous bacille Calmette-Guérin vaccination, but definitive data to quantify the risk posed by previous bacille Calmette-Guérin vaccination or the benefit of INH chemoprophylaxis are scant. INH chemoprophylaxis can be withheld if there is negative QuantiFERON-TB Gold assay for a transplant candidate with previous vaccination [45].

Chemoprophylaxis with INH should be given prior to transplant for all TST-positive or Quantiferon-positive candidates. In randomized trials in transplant recipients from India and Pakistan, INH prophylaxis effectively prevented tuberculosis [52–54]. One report from Turkey indicated that none of the 77 HCT patients treated with INH developed active tubercular infection, while five of the 274 other patients studied developed active peritransplantation mycobacterial infection [55]. Programs in Pakistan [53] and in Turkey [55] give INH to all TST-negative HCT recipients for 6 months, with a reduction in the development of active tuberculosis. Specifically, in liver transplant candidates at risk for infection after transplantation, INH chemoprophylaxis used during candidacy does not adversely affect hepatic function [56, 57].

INH chemoprophylaxis that starts before transplantation is continued to complete 6–12 total months continuous therapy [58]. The course can be completed prior to transplant if possible, but it is not a contraindication to proceed with transplantation while completing therapy [49, 59]. It is more practical to complete this course before the transplantation period for renal transplant candidates on renal replacement therapy [60], where there are prolonged wait list times. INH prophylaxis is safe for renal, lung and liver transplant candidates, and it would probably be effective prior to heart transplantation as well [52, 56, 61, 62].

Potential problems with INH therapy include liver toxicity (drug-induced hepatitis, especially in older individuals), neuropathy, drug interactions with medications such as cyclosporine, and the possibility of selecting out INH-resistant organisms. INH does not affect the bioavailability of cyclosporine in renal transplant recipients such that dosing changes are necessary [63]. INH therapy may be interrupted in transplant recipients who develop liver function abnormalities, a particularly common problem in the HCT and liver transplant settings. Tuberculosis prophylaxis with levofloxacin in liver transplant patients is associated with tenosynovitis [64].

Donor screening is performed in countries where tuberculosis is endemic. A report from Turkey found a 23 % rate of TST reactivity among both HCT donors and intended recipients [49]. A report from a Mexican program notes screening of all potential donors [65].

Transmission of tuberculosis by the transplanted organ, although rare, has also been documented [66–69]. Two separate cases of multidrug-resistant tuberculosis occurred in lung transplant recipients who probably acquired the disease from the donor [66, 67]; one of these cases required pulmonary resection for control of the infection [67]. Quinibi et al. suggest INH prophylaxis for TST-negative recipients of organs from TST-positive donors [40].

One report of tuberculous meningitis following travel to an endemic area 4 years after transplant suggests prophylaxis during travel [70]. This cannot be substantiated by other cases.

22.5 Hematopoietic Stem Cell Transplants

22.5.1 Epidemiology

Patients receiving allogeneic or autologous HCT are severely immunocompromised because of their underlying condition, the pretransplantation chemotherapy and radiation therapy and by the later development of GVHD and its prolonged, intensive immunosuppressive therapy. A variety of typical and atypical mycobacterial infections have been described in these patients [71–85]. However, even though the literature describing mycobacterial infections in HCT recipients is small, studies have not shown a high incidence of mycobacterial infections (summarized in Table 22-1). There is no information regarding the relative impact of stem cell source and conditioning regimen on risk.

Navari et al. reported seven mycobacterial infections (approximately 1 %) in their series of 682 patients with acute leukemia who received allogeneic bone marrow grafts [74]. Four pulmonary and three extrapulmonary infections with *M. tuberculosis* or atypical mycobacteria were observed. In a report from the M. D. Anderson Hospital that was published in 1984, Kurzrock et al. reported three patients (3.3 %) with mycobacterial infections in a series of 90 allogeneic bone marrow transplantations for hematologic malignancies or aplastic anemia [83]. Two infections were with *M. tuberculosis*, and one was with *Mycobacterium avium-intracellulare* complex. The two patients with *M. tuberculosis* infections were from Latin America, a high prevalence area for tuberculosis. The authors of this report emphasized the difficulties in making a specific diagnosis due to the unusual presentations, which often involved more than one pathogen.

A study from the University of Minnesota reviewed the experience with mycobacterial infections after bone marrow transplantation over a 20-year period [73]. Eleven mycobacterial infections (0.49 %) were diagnosed in 2241 recipients of allogeneic (9 of 1486) or autologous (2 of 755) HCTs. Two patients with *M. tuberculosis*, two with *M. avium-intracellulare complex*, and seven with infections caused by rapidly growing, atypical mycobacteria (e.g., *Mycobacterium fortuitum* or *Mycobacterium chelonae*) were described.

Other reports describing tuberculosis in allotransplant recipients from areas where the infection is more endemic have been reported. Tuberculosis develops in 1.4–3.5 % of allograft recipients, both pulmonary and extrapulmonary [77–79, 85]. In China, tuberculosis infections were diagnosed in 9 (3.1 %) of the 295 transplant recipients, from 45 to 165 days after transplantation [78]. Multivariate analysis revealed that a previous history of tuberculosis and total body irradiation increased the risk of tuberculosis in HCT patients (relative risk, 4.8 and 12.5, respectively) [78]. In Taiwan, while 1.9 % of HCT recipients developed newly diagnosed TB, the 10-year cumulative incidence was 3.5 %, and those with TB had a higher mortality rate than recipients without TB [85]. Independent risk factors for TB included adult age and GVHD [85].

Several additional case reports of both typical and atypical mycobacterial infections in HCT recipients have been published [15, 51, 55, 72, 76, 79, 82, 84, 86–90]. These reports emphasize the low incidence and the variety of unusual presentations of mycobacterial infection in HCT recipients and the generally successful treatment outcome with appropriate therapy. Even in Turkey, where the prevalence of tuberculosis is much higher, HCT recipients have relatively few active infections, especially with the judicious use of INH prophylaxis [55].

Atypical mycobacterial infection prior to transplant is not necessarily a contraindication to transplant [91]. A patient with chronic myeloid leukemia who developed a disseminated infection involving the liver with *Mycobacterium avium* complex was successfully treated 2 years prior to transplant. The patient underwent allogeneic bone marrow transplantation from a human leukocyte antigen (HLA)-matched sibling donor. The antimycobacterial prophylaxis given during transplant included ciprofloxacin and clarithromycin to day 100.

22.5.2 Clinical Manifestations and Diagnosis

Atypical presentations make the recognition of mycobacterial infections in HCT recipients difficult. These patients are severely immunocompromised and often neutropenic, which

Table 22-1. Selected reports of mycobacterial infections after hematopoietic stem cell transplantation

Reference	Number of patients	Mycobacterial infections	Country	Sex (M/F)	Transplant type	Organism		Site of infection		Interval from transplant	Comorbidity at time of infection	Infection outcome
						Mycobacterium tuberculosis	Atypical mycobacteria	Pulmonary only	Non-pulmonary or disseminated			
de la Camara et al. [71]	8013	20 (0.25 %) (0.25 % TB)	Spain	9/11	12/2866 Allo 8/5147 Auto	20	0	16	4	1–111 months	All allos had GVHD	17 Resolved
Gaviria et al. [72]	6259	40 (0.6 %)	USA	20/20	28 Allo, 12 auto	3	40	15	25 (23 Catheter related; 2 lymphaden-itis)	Catheter related; 2 months (1–12 months); other: 9 months (1–40 months)	Copathogens: 3 fungal, 4 viral; 6 bacterial, 3 neutropenia, 21 GVHD, 18 steroids	All resolved
Cordonnier et al. [51]	4525	31 (0.7 %) (0.4 % TB)	EBMT	15/16	23/1513 Allo 8/3012 Auto	20	8 (+3 Granulomas)	17	14	9 months median	24 GHVD	26 Survived
Roy and Weisdorf [73]	2241	11 (0.5 %) (TB 0.09 %)	USA	6/5	9/1468 Allo, 2/755 auto	2 (Both allo)	9 (7 Allo, 2 auto)	2	9	<100 days	5 GVHD, prolonged neutropenia±bacterial infection	9 of 9 treated, resolved
Navari et al. [74]	682	7 (1.0 %) (TB 0.6 %)	USA	5/2	682 Allo	2 (+2 Lung granulomas)	3	4	3	2–3 months	NR	6 of 6 treated, resolved
Aljurf et al. [75]	641	4 (0.1 % TB)	Saudi Arabia	2/2	Allo	4	0	2	2	4–20 months	1 Graft failure, 2 viral infections	2 of 4 treated, resolved
Martino et al. [76]	355	2 (0.56 %)	Spain	NA	2/118 Allo 0/237 Auto	2	0	1	1	4–19 months		2 Survived
Budak-Alpdogan et al. [55]	351	5 (1.4 %)	Turkey	4/1	351 Allo	5	0	4	1	12 months (10–47 months)	4 GVHD	All resolved
Ku et al. [77]	350	8 (2.3 %)	Taiwan	6/2	8/255 Allo 0/95 Auto	8	0	8	0	3.5 months (1–33 months)	7 GVHD	3 Resolved; 4 died (all had active TB)
Lee et al. [78]	295	9 (3.1 %)	Korea	NA	NA	9	0	NA	NA	1.5–5.5 months	NA	NA
George et al. [79]	217	3 (1.4 %)	India	3/0	217 Allo	3	0	0	3	1, 3, 15 months	2 GVHD	2 of 3 treated, both survived

(continued)

TABLE 22-1. (continued)

Reference	Number of patients	Mycobacterial infections	Country	Sex (M/F)	Transplant type	Organism		Site of infection		Interval from transplant	Comorbidity at time of infection	Infection outcome
						Mycobacterium tuberculosis	Atypical mycobacteria	Pulmonary only	Non-pulmonary or disseminated			
Ip et al. [80]	183	10 (5.5%)	Hong Kong	4/6	NR	10	0	10	0	1–18 months	7 Acute GVHD, 5 chronic GHVD	7 Resolved
Ullah et al. [81]	154	4 (2.6%)	Pakistan	3/1	154 Allo	4	0	3	1	1–12 months	Pleural effusions	3 of 4 treated resolved
Unal et al. [82]	132	5 (3.8%)	USA	3/2	105 Allo 64 Auto	0	5	2	3	0.5–9 months	4 Alemtuzumab, 1 ATG	4 Resolved
Kurzrock et al. [83]	90	3 (3.3%) (TB 2.2%)	North America	3/0	90 Allo	2	1	3	0	2–11 months	All had acute or chronic GVHD	2 Died
Eom et al. [84]	2841	13 Pretransplant	Korea	8/5	NA	9	0	8	5	Prior to transplant	1 Developed meningitis	4 Died
Fan et al. [85]	2040	39 (1.9%)	Taiwan	27/12	32/1368 Allo 7/672 Auto	39	0	34	5	Median 15 months	cGVHD	20 Died

Allo allogeneic, *ATG* antithymocyte globulin, *auto* autologous, *EBMT* European Blood and Marrow Transplant Group, *GVHD* graft-versus-host disease, *INH* isoniazid (isonicotinic acid hydrazide), *M/F* male/female, *NA* not available, *NR* not reported.

can mask a febrile response and can obscure a granulomatous reaction. The presenting features are nonspecific, and they are related to the site of infection. In a review of mycobacterial infections in 2241 transplant recipients over a 20-year period, the clinical manifestations of mycobacterial infection included unexplained fever, pulmonary infiltrates, osteomyelitis, and central venous catheter tunnel inflammation [73]. The most common manifestation of mycobacterial infection in these patients was central venous catheter-related infection, which occurred in 6 of 11 patients. Atypical, rapidly growing mycobacteria caused all of the central venous catheter–related infections. Reports from other blood and marrow transplantation centers have described a similar spectrum of mycobacterial infectious syndromes [15, 55, 72, 76, 79, 83, 84, 86, 88, 92]. A large series from Seattle indicated that less than 1 % of patients were infected with atypical mycobacteria, with nearly all (21 of 23) experiencing catheter-related infections that resolved with therapy and removal of the catheter [72].

Invasive procedures may be required to obtain diagnostic material for culture or histopathology. Because the laboratory diagnosis of mycobacterial infection requires specific cultures that are often held for prolonged periods [4, 5], a high index of suspicion is required to request the appropriate diagnostic studies specifically to identify these infections. HCT recipients often have multiple coexisting infectious pathogens that can mask the existence or the significance of mycobacterial isolates. Although mycobacterial infections are uncommon in HCT recipients, they should be considered in all apparently affected HCT recipients, particularly those at high risk because of previous tuberculosis exposure, a positive TST or QuantiFERON-TB Gold test, residence in endemic area, or ethnic background.

22.6 Solid Organ Transplants

22.6.1 Immune Defects in Solid Organ Transplant Recipients

Several factors contribute to the incidence of infection in solid organ transplant recipients [93–124]. These patients are often immunosuppressed because of their underlying condition (e.g., uremia, liver failure, or malnutrition). In addition, therapeutic immunosuppression to prevent graft rejection is lifelong and is probably the most important factor contributing to infection in transplant recipients. The risk is likely exaggerated in those requiring boosted immunosuppression for episodes of rejection.

22.6.2 Epidemiology

Mycobacterial infections are more common in recipients of solid organ transplant than stem cell transplant (Table 22-2). In contrast to HCT recipients, solid organ transplant recipients typically remain immunosuppressed for long periods or for life. Consistent with this is the observation that mycobacterial infections generally occur later and more frequently in organ transplant recipients compared with patients who receive HCTs. Infections were diagnosed a mean of 48 months following transplantation, with rare infections being reported as late as 269 months after transplantation [108, 109]. In some, but not all, reports from heart transplant recipients, early infections have been noted [104, 110]. This contrasts with the experience in HCT recipients, in whom many infections occur early in the course of the transplantation [73]. A higher incidence of *M. tuberculosis* and nontubercular mycobacterial infections has been reported in renal transplant recipients than in the general population [125]. Three US renal transplantation centers [105, 106, 120] reported a 0.5–0.7 % incidence of tuberculosis, compared with that of 0.01 % in the general population [126]. In contrast, centers in areas endemic for tuberculosis report much higher incidences that range from 3.5 to 11.8 % [40, 93, 98, 100, 107]. Patients undergoing liver or heart transplantation also have higher rates of mycobacterial infections than do the general population; the reported incidences varied between 0.5 and 2.7 % [28, 39, 104, 127–129].

Nontuberculous mycobacterial infections after solid organ transplantation have been published mainly as case reports, which prevent a reliable estimation of the incidence of such infections in these patients. These are usually caused by *M. avium-intracellulare* complex [102, 103, 112], *Mycobacterium kansasii* [94, 102], *M. abscessus* [130], *M. chelonae* [103], and *M. fortuitum* [94, 131] and, rarely, by other species [116, 130, 132–134]. Their diagnosis is most frequent within the first year after transplantation [135], but it can be as late as 11 years after transplantation (mean of 3.5 years) [121, 136]. Patients present with isolated pulmonary infections, cutaneous lesions, tenosynovitis, joint infections, empyema, enteritis, or surgical wound infection.

22.6.3 Clinical Manifestations and Diagnosis

Tuberculosis and atypical mycobacterial infections often demonstrate unusual features in immunocompromised patients. Patients often present with nonlocalized, systemic symptoms. Studies of tuberculosis in organ transplant recipients consistently show a high proportion of patients—as many as 63 %—with nonpulmonary or disseminated tuberculosis [16, 40]. Extrapulmonary presentations include meningitis [39, 98, 99], peritonitis [114], lymphadenopathy [99], liver abscess [39, 111], disseminated intravascular coagulation [39], pericarditis [137], cutaneous abscesses [138], and renal tuberculosis. In one study, 20 % of renal transplant recipients had a fever of unknown origin [100]. Some patients may be asymptomatic, and the infection may be diagnosed incidentally or, rarely, at the postmortem examination [107].

Because of nonspecific clinical manifestations, the diagnosis of mycobacterial infections in organ transplant recipients can be difficult. Patients receiving immunosuppressive therapy

Table 22-2. Selected reports of mycobacterial infections after solid organ transplantation

Reference	Number of patients	Mycobacterial infections	Country	Sex (M/F)	Transplant type	Organism		Site of infection		Interval from transplant	Comorbidity at time of infection	Infection outcome
						Mycobacterium tuberculosis	Atypical mycobacteria	Pulmonary only	Non-pulmonary or disseminated			
Ghafari et al. [93]	1350	52 (3.9 %)	Iran	40/12	Renal	52	0	36	16	4–140 months	37 % Graft loss	12 D
Quiepo et al. [94]	1261	27 (2.1 %)	Spain	NA	Renal	20	7	17	8	20.5 months	NA	4 D
Sayiner et al. [95]	880	36 (4.1 %)	Turkey	29/7	Renal	36	0	19	16	3–111 months	NR	8 D
Spence et al. [96]	565	5 (0.9 %)	USA	2/3	Renal	3	2	2	3	NR	3 Rejections	5 Survived, 4 drug toxicity
Meyers et al. [39]	550	5 (0.9 %)	USA	3/2	Liver	5	0	1	4	2–57 months	4 Rejections	2 D, 3 R
Yildiz et al. [97]	520	22 (4.2 %)	Turkey	19/3	Renal	22	0	12	10	44 months	2 Kaposi, 2 dialysis	6 D
Novick et al. [28]	502	14 (2.8 %)	USA	13/1	Heart	0	14	4	10	86 Days to 11.5 years	Increased incidence of rejection episodes	1 D, 12/12 treated R, 1 NT
Hall et al. [98][a]	487	22 (4.5 %)	South Africa	14/7[b]	Renal	21	1	16	5	14 months (2–74 months)	9 (Recent increase in immuno-suppression)	1 NT, 1 D, 3 URD, 12 R, 5 NR
Qunibi et al. [40][a]	403	14 (3.5 %)	Saudi Arabia	11/3	Renal	14	0	4	10	1–84	NR	12 R, 1 D, 1 URD
Kaaroud et al. [99]	359	9 (2.5 %)	Tunisia	7/2	Renal	9	0	8	1	49.6 months	TST negative	2 D
Sakhuja et al. [100][a]	305	36 (11.8 %)	India	31/5	Renal	36	0	15	14 (+7 FUO)	1 year	NR	2 D, 5 URD, 18 R, 11 LFT
Apaydin et al. [101]	274	16 (5.8 %)	Turkey	14/2	Renal	16	0	13	3	4–119 months	1 Dialysis	6 D
Malouf and Glanville [102]	261	23 (9 %)	Australia	11/12	Lung, heart-lung	2	21	18	5	22 months (0.1–100 months)	30 % Coincident rejection	50 % Improved lung function; 72 % survival; no D
Kesten and Chapparo [103]	219	8 (3.8 %)	USA	5/3	Lung	2	6	5 Alveolar lavage	1	NR	NR	All responded

Munoz et al. [104]	144	3 (2 %)	Spain	2/1	Heart	3	0	2	1	50–102 days	2 Acute rejection	3 R
Denaly et al. [105]	1097	10 (0.9 %)	USA	NR	Renal	6	4	4	6	NR	1 HIV+; 4 acute rejection	7 R, 3 URD
Lloveras et al. [106]	106	7 (6.5 %)	USA	6/1	Renal	4	3	1	6	20 (3–38 months)	NR	All responded, 1 D
Malhotra et al. [107][a]	95	9 (9.5 %)	England	9/0	Renal	9	0	5	4	2–32 months	NR	2 PMDx, 2 URD, 5 R
Patel et al. [108]	4	4	USA	4/0	Renal, heart, lung	0	4	1	3	6 Years (1.3–11 year)	NR	All responded
Fairhurst et al. [109]	1	NR	USA	0/1	Heart	0	1	1	1	64 months	EBV lymphoma	Responded

D death related to mycobacterial infections, *FUO* fever of unknown origin, *HIV* human immunodeficiency virus, *LTF* lost to follow-up, *M/F* male/female, *NR* not reported, *NT* not treated, *PMDx* postmortem diagnosis, *R* resolved, *URD* death unrelated to mycobacterial infection.

[a]Report from an area of high prevalence of tuberculosis.

[b]*M. tuberculosis* only reported.

[c]Not speciated.

have a blunted inflammatory response, so the characteristic granulomatous reaction to mycobacteria may not be seen. Patients may often have coexistent infectious and noninfectious complications that can add to the difficulty in diagnosis [40, 104]. A high index of suspicion is therefore crucial to performing an appropriate diagnostic workup and requesting mycobacterial cultures. Invasive procedures may often be necessary to obtain diagnostic material [98, 108, 139].

Patients who develop liver failure from INH may go on to orthotopic liver transplantation, and will require the remainder of their antituberculous treatment course in the months immediately following transplantation. In three cases, liver transplantation performed for progressive hepatic failure was successful [140, 141]. Even multidrug resistance was not a contraindication to liver transplant [141]. A modified antituberculous drug regimen was used while taking standard doses of immunosuppressive drugs.

22.6.4 Treatment

As in the general population, the treatment of tuberculosis in transplant recipients involves therapy with combination antimycobacterial agents [142]. Most centers use a combination of INH with or without rifampin for at least 12 months. One or two additional drugs, such as ethambutol or pyrazinamide, are often included in the treatment program for the first 2–3 months. Despite continuing immunosuppression, reports indicate that HCT recipients with tuberculosis respond to standard antimycobacterial therapy, but extensive data on the clinical and bacteriologic responses are not available.

The optimal duration of antituberculous therapy in these patients is controversial. Therapy is usually given for 12–18 months [142]. Limited experience suggests that a shorter treatment duration, similar to therapy in immunocompetent hosts, may be adequate. Successful outcomes with a 9-months regimen have been recorded in renal transplant recipients with localized pulmonary tuberculosis [100, 102, 123, 143].

Some drugs used to treat tuberculosis can have important side effects and drug interactions in HCT recipients. INH may cause transplant-related liver toxicity, and it must often be discontinued in the immediate posttransplantation period. Rifampin, a hepatic cytochrome P-450 enzyme inducer, can accelerate cyclosporine metabolism, resulting in subtherapeutic drug levels and increasing the risk of GVHD or graft rejection [144]. In posttransplant patients, rifampin-containing regimens are often avoided. Comparative efficacy data on which recommendations regarding the choice of therapy of tuberculosis in organ graft recipients to base are unavailable. The clinical setting, the frequency of drug resistance in the community, and in vitro sensitivities should guide therapy, but nonrifampin-containing regimens generally need to be administered for longer periods [119].

Multidrug-resistant tuberculosis occurs in several endemic areas around the world, including the USA and Europe. A case of multidrug-resistant tuberculosis in a Chinese double-lung transplant recipient was probably acquired from the donor [66]. Tenosynovitis was noted in a heart transplant recipient in France [145]. Reactivation of pulmonary tuberculosis acquired 10 years prior to heart transplant, while the patient was incarcerated [146], was resistant to both INH and rifampin during susceptibility testing. A report of drug-resistant tuberculosis in a bone marrow transplant recipient had a good result to second line therapy [147]. A renal transplantation center in India reported a high incidence of primary drug-resistant tuberculosis [148]. INH resistance was seen in 7 of 23 and rifampin resistance in 5 of 23 M. *tuberculosis* isolates from 727 renal allograft recipients [148]. This suggests that multidrug-resistant infections are likely in patients who come from communities harboring a large reservoir of drug-resistant *M. tuberculosis*. Patients residing in areas where drug-resistant tuberculosis rates are high should begin treatment with four antituberculous drugs, such as INH, rifampin, ethambutol, and pyrazinamide, until antimicrobial susceptibility results are available. The duration of therapy should be prolonged for 18–24 months in patients with drug-resistant tuberculosis.

Treatment of atypical mycobacterial infections is dictated by the identified species. Coverage with a macrolide and a fluoroquinolone is used until susceptibilities are ready. Remembering that atypical mycobacteria are often not susceptible to the agents typically used to treat tuberculosis is important. Furthermore, the drug susceptibilities of atypical mycobacteria are often unpredictable. Therefore, in vitro drug sensitivity assays should always be performed, and treatment should be revised if resistance to any of the drugs being used is demonstrated.

In general, antituberculous therapy in transplant recipients is effective despite continuing immunosuppression. However, mortality from tuberculous infections in transplant recipients has been reported. The role of temporarily reducing immunosuppression to facilitate antituberculous response has been discussed in the literature without achieving a consensus. Some authors recommend routinely decreasing immunosuppression until febrile illness resolves [110], yet this is rarely done in heart or lung transplant recipients who need higher immunosuppressive drug dosing, particularly in the early posttransplant period. Several investigators, however, report successful treatment of tuberculosis despite continuing immunosuppression.

22.6.5 Secondary Prophylaxis

Because of the lifelong immunosuppression in solid organ transplant recipients, posttreatment prophylaxis with INH has been proposed by some investigators [98]. However, reports of successful treatment in most patients, without a significant incidence of late recurrence, argue against the need for posttreatment prophylaxis. In the liver transplant population, a history

of tuberculosis treated prior to transplant did not lead to reactivation among the 6 of 1116 liver transplant recipients, who were observed for a median of 25.5 months (range 12–82). This study found no rationale for INH prophylaxis in liver transplant recipients with past diagnosis of tuberculosis, when the disease was considered to be inactive [149].

22.7 Conclusion

A clinical strategy of aggressive surveillance in high-risk patients, maintenance of a high index of suspicion, early diagnosis, and prompt treatment is most likely to be effective in limiting morbidity and mortality from mycobacterial infections in transplant recipients.

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23
Other Bacterial Infections After Hematopoietic Stem Cell or Solid Organ Transplantation

Lynne Strasfeld and Stephen Dummer

23.1 Introduction

This chapter describes the epidemiology, clinical presentation, diagnosis, and management of infections caused by a diverse group of bacterial pathogens. These include classic opportunistic infections and also infections that are common in immunocompetent patients, but particularly prevalent or morbid in transplant populations. Some uncommon bacterial pathogens that have a predilection for patients with impaired immunity are discussed. Finally, the chapter touches on some miscellaneous bacterial infections that are important because they may be unanticipated in transplant recipients or present diagnostic or therapeutic challenges.

23.2 Gram-Positive Organisms

23.2.1 Listeria Monocytogenes

Listeria are small gram-positive bacilli that produce weak beta hemolysis on blood agar and have characteristic tumbling motility when observed by light microscopy [1]. Isolation from mixed specimens such as stool requires special media or a process called "cold enrichment" that capitalizes on the ability of *Listeria* to grow well at refrigerator temperatures. In clinical specimens, the organisms may appear gram-variable or resemble diphtheroids. Indeed, isolation of a "diptheroid" from the blood or CSF should raise concern for laboratory misidentification of *Listeria*.

Of the seven species of *Listeria*, only one, *L. monocytogenes,* is responsible for almost all cases of listeriosis. This organism is widespread in nature and has been isolated from tap water, sewage, several animals, and multiple foodstuffs including dairy products, fruits, vegetables, fish, and meats [1]. Human exposure to *Listeria* appears to be universal. Gastrointestinal carriage has been documented in about 5% of healthy adults and asymptomatic kidney transplant recipients [1–3].

Excepting perinatal transmission and rare cases of person-to-person spread, *Listeria* infections are thought to be acquired by ingestion of contaminated food [4]. High profile outbreaks [4, 5] have highlighted the foodborne nature of this infection, but most sporadic cases have no identified food source. The incubation period for self-limited cases of febrile gastroenteritis is about 24 h, while the incubation period of invasive infection averages 35 days, with a range of 1–91 days [5, 6]. Invasive listeriosis occurs predominately in four risk groups: immunocompromised individuals, pregnant women, infants, and adults over 60 years of age. Three quarters of the 1651 of patients identified with listeriosis in the USA between 2009 and 2011 who did not have age or pregnancy as a risk factor were immunocompromised [7]. Recent surveillance data indicate that the incidence of both non-perinatal *Listeria* infection and *Listeria*-associated mortality are decreasing, trends that are likely due to improved control mechanisms in the food industry [8].

Experiments by Mackaness in the 1960s demonstrated the central role of cell-mediated immunity in protection against *Listeria* infection [9]. Multiple arms of the immune response are involved, but memory CD8 T cells seem to be most important to protection. *Listeria* infection has been reported after both solid organ transplantation (SOT) and hematopoietic cell transplantation (HCT). The risk for invasive listeriosis is highest early after transplantation or following augmentation of immune suppression [10, 11]. Some early posttransplant *Listeria* infections are postulated to arise by translocation from pretransplant gut carriage [12, 13]. *Listeria* infections also occur years after transplantation, when immunosuppression is generally less intensive [12]. Recent 7-year data from France showed an incidence of *Listeria* infection of 7.91 cases /100,000 persons/year in SOT recipients, which was 21 times higher than in the general population; the mortality rate in transplant patients was 6% [14].

In transplant recipients, listeriosis typically presents as a sepsis syndrome, often accompanied by central nervous system (CNS) involvement. The presentation can be acute or can follow a prodrome of milder symptoms. Almost all transplant recipients with listeriosis have bacteremia and between 40 and 60% have meningitis [11, 15]. Signs of CNS

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involvement may be subtle, and nuchal rigidity is present in only about one-half of patients [1]. Focal neurologic signs are less common than diffuse signs, such as personality changes or forgetfulness.

In the largest series of *Listeria* meningitis, the median cerebrospinal fluid (CSF) leukocyte count was 585 cells/mm^3 and nearly 70% of patients had <1000 leukocytes/mm^3 [16]. CSF smears typically showed a predominance of neutrophils. Elevated CSF protein and low CSF glucose levels were common, although these values were sometimes normal, particularly early in the illness. The organism is only occasionally visualized on Gram stains of CSF, but centrifugation of CSF may increase the yield. Cultures of CSF are relatively insensitive, identifying only 30–35% of cases [16, 17].

In addition to meningitis, *L. monocytogenes* can cause cerebritis, encephalitis, and brain abscess (see Figure 23-1). Rhombencephalitis is an unusual form of listerial encephalitis involving the brainstem. It presents with movement disorders, facial nerve palsies, cerebellar signs, and hemiparesis or hemisensory deficits. *Listeria* brain abscesses often involve subcortical areas, including the brain stem. Most patients with *Listeria* brain abscess are bacteremic, and 10–25% have meningitis [1, 11, 16, 17]. The mortality of isolated bacteremia with *L. monocytogenes* is only 3%, but is as high as 30% in patients with CNS involvement [11, 15–17]. Non-CNS *Listeria* also occasionally causes localized infection in transplant recipients, including peritonitis [18], hepatitis [19], arthritis [20], endophthalmitis [21], and endocarditis [22].

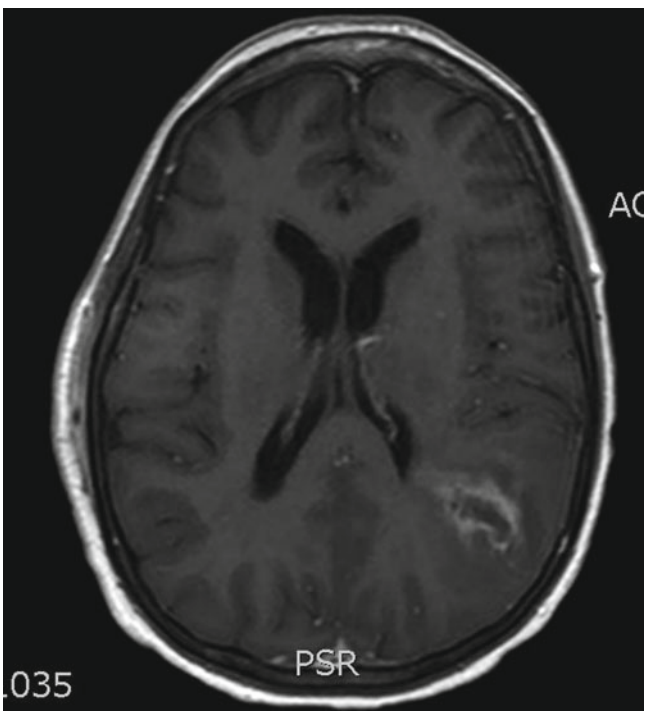

FIGURE 23-1. MRI demonstrating ill-defined enhancing focus with surrounding vasogenic-type edema in the juxtacortical left parietal lobe.

Lacking controlled studies, recommendations for treatment of listeriosis are based on in vitro testing, animal models, and clinical observation. The therapy of choice is high-dose ampicillin or penicillin [1, 17]. Laboratory evidence of synergy between ampicillin and aminoglycosides has led to the recommendation for combination therapy when infection is severe or occurs in immunocompromised hosts [23]. In transplant recipients, however, the potential benefit of aminoglycoside therapy must be weighed against the risk of nephrotoxicity. Trimethoprim-sulfamethoxazole (TMP-SMX) is the drug of choice in penicillin-allergic patients [1]. Imipenem and meropenem have been successfully used to treat *Listeria* infections, but they are generally less active than ampicillin and may lower the seizure threshold [24, 25]. Linezolid can be considered for patients with multiple drug allergies based on its excellent CNS penetration and effectiveness in a few clinical cases [26]. Vancomycin has good in vitro activity against *Listeria* but suffers from poor penetration into the CNS; clinical failure for both CNS and non-CNS listeriosis has been reported with vancomycin [27, 28]. Cephalosporins are inactive against *Listeria*. Due to the high risk of recurrence, transplant recipients should receive 3 weeks of therapy for bacteremia or meningitis, and longer courses for brain abscess.

Guidelines for preventing *Listeria* infection from the Centers for Disease Control and Prevention (CDC) include standard approaches to food safety, such as thorough cooking of meat, washing of fresh fruits and vegetables, and physical separation of uncooked meat from other foods [29]. Persons at high risk of listeriosis are encouraged to avoid foods that may harbor *Listeria*, including unpasteurized milk, soft cheeses, hot dogs, and luncheon meats. Standard sulfonamide prophylaxis for *Pneumocystis* infection is thought to prevent *Listeria* infection but the low incidence of infection has made this difficult to prove [1, 17].

23.2.2 Nocardia

Nocardia are aerobic gram-positive rods that have characteristic filamentous, branching chains (see Figure 23-2a). They are present in soil and decaying organic material, and most human infections result from the inhalation of airborne bacilli. A small number of patients are infected by accidental inoculation into the skin. *Nocardia* infection was first described in transplant recipients in the 1960s [30]. *Nocardia* infections occur in between 0.1 and 3.5% of SOT patients, with lung and heart recipients at highest risk [31, 32]. The reported frequency of nocardiosis after HCT is low. An incidence of 0.3% was reported in 6759 HCT recipients at three large transplant centers [33]; cases occurred exclusively in allogeneic recipients. In a separate single-center study, nocardial infection occurred in 1.7% of 302 allogeneic HCT recipients but only 0.2% of 542 autologous recipients [34];

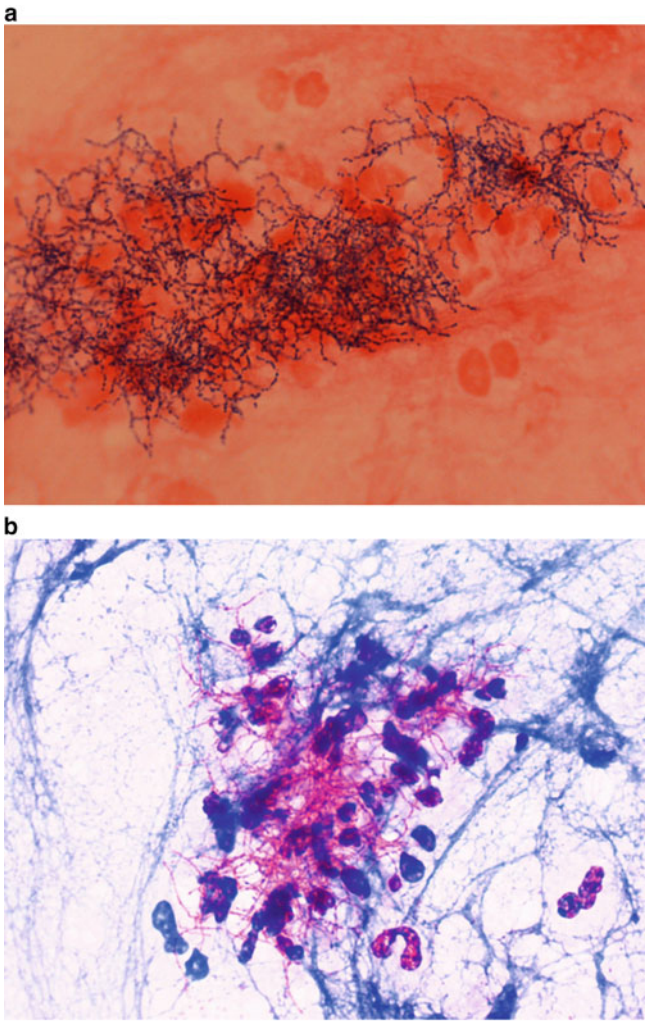

FIGURE 23-2. (**a**) Branching, beaded filamentous rods (hematoxylin and eosin stain). (**b**) Modified acid fast stain, demonstrating the weakly acid fast staining property of *Nocardia* species.

all patients with *Nocardia* infection had recently received immunosuppressive medications.

Nocardia infections are usually sporadic and acquired in the outpatient setting, but small nosocomial outbreaks have been reported [35]. Wilson and coauthors [30] noted few *Nocardia* infections in the first month after SOT; the frequency of cases peaked between 1 and 6 months after transplantation and then occurred sporadically at lower rates thereafter. Similarly, most cases of nocardiosis in HCT recipients occur after engraftment but within the first post-transplant year [33, 34]. Receipt of high-dose corticosteroids in the previous 6 months, high blood levels of calcineurin inhibitors and a history of cytomegalovirus disease have been shown to be independent risk factors for *Nocardia* infection in SOT recipients [32].

The clinical manifestations of nocardiosis are similar in SOT and HCT recipients [30–34]. Eighty percent to 90% of patients have a respiratory illness, ongoing for a week or more. Typical symptoms are fever, productive cough, pleuritic chest pain, dyspnea, weight loss, and hemoptysis. Lung nodules, which may be cavitary, are the classic radiographic finding, but alveolar consolidation and/or pleural effusion are also seen [36]. At presentation, up to one-half of patients have disseminated infection. Sites of dissemination include the CNS in roughly one-third of patients and the skin in up to 15%. Occasional patients have spread to the bone, joints or muscle [30–33]. Skin lesions of disseminated nocardiosis are palpable, mildly tender, deep abscesses that may or may not appear erythematous. Cerebral abscesses are the usual manifestation of CNS infection. They may manifest with focal neurologic defects, headache, and/or seizures [30]. Some brain abscesses are clinically silent. Given the relative frequency of CNS involvement, neuroimaging is advised in all patients with nocardiosis. Meningitis due to *Nocardia* also occurs but is considerably less common than brain abscess.

The gold standard for diagnosis of *Nocardia* infection is culture of the organism from a clinical specimen. Biopsy of lung, brain, or other involved tissue is often necessary. *Nocardia* colonies may appear on aerobic cultures as early as 3–5 days, but can take 2 weeks or longer to be detected. In a study of 11 lung transplant recipients with *Nocardia* infection, the mean time of culture diagnosis was 9 days [37]. The appearance of *Nocardia* on Gram stain (see Figure 23-2a) is distinctive enough to allow for a presumptive early diagnosis. Most strains of *Nocardia* are weakly acid fast (see Figure 23-2b), a feature that aids in identification and in differentiation from *Actinomyces* species.

Sulfonamides are the agents of choice for nocardiosis because of their reliable activity and the high drug concentrations achieved in affected tissues. Trimethoprim-sulfamethoxazole is the preferred sulfonamide, but excellent results also have been achieved with other sulfonamides [30, 34, 38, 39]. Adjustment of sulfonamide dosing for creatinine clearance is often necessary in transplant patients. Ideal serum levels are between 100 and 150 mcg/mL. Clinical failure of sulfonamide therapy is uncommon. A high rate of in vitro resistance of *Nocardia* isolates to sulfonamides was reported in one recent study [40]. However, a subsequent study from six reference laboratories showed resistance to TMP-SMX in only 2 of 552 *Nocardia* isolates [41]. Other antimicrobials that have good activity against most species are minocycline, amikacin, imipenem, meropenem, cefotaxime, and ceftriaxone [31]. A recent in vitro study demonstrated that tigecycline and moxifloxacin were active against the majority of 51 clinical isolates of *Nocardia* [42]. Selected strains are susceptible to ampicillin, ampicillin-clavulanate, ciprofloxacin, erythromycin, and other macrolides, but use of these antibiotics is only advised if supported by susceptibility testing. Ertapenem is 16-fold less active than imipenem and should not be viewed as a useful agent [42]. Linezolid has excellent in vitro activity against *Nocardia* species and has demonstrated therapeutic potential in case reports [43, 44]. Unfortunately, the long-term use of linezolid may

be limited by adverse effects such as myelosuppression and peripheral neuropathy.

Susceptibility testing of *Nocardia* species has not been rigorously correlated with clinical outcomes, but offers comparative data and may be helpful with more resistant strains, such as *N. farcinica* or *N. transvalensis*, or if therapy must be changed from a first-line agent because of toxicity or inadequate response [42, 45].

Animal models have demonstrated that certain antimicrobial combinations, such as imipenem with amikacin or moxifloxacin, may achieve more rapid reduction in bacterial colony counts than sulfonamides [46–48]. These regimens may be an excellent alternative to sulfonamides or serve as initial therapy until clinical stability is achieved and susceptibility data is available. Ultimately, most patients respond to initial therapy and can be transitioned to a simple oral regimen to complete treatment. The optimal duration of therapy is unknown, but treatment courses of 4–6 months are typical for pulmonary and soft tissue infections. Treatment is usually extended to 12 months or longer in patients who have disseminated or CNS disease.

Although TMP-SMX is generally an active agent for treatment of nocardiosis, low-dose TMP-SMX prophylaxis for *Pneumocystis* is not consistently protective against *Nocardia* infection [32–34, 37]. Interestingly, isolates from patients who develop nocardiosis while on low-dose TMP-SMX are usually susceptible to TMP-SMX [32].

23.2.3 Lactobacilli

Lactobacilli are strict or facultatively anaerobic, gram-positive rods that are ubiquitous inhabitants of the human oral cavity, vagina, and gastrointestinal tract. Previously, they have often been considered nonpathogenic. However, serious infections due to lactobacilli have been reported in both immunosuppressed and immunocompetent patient populations [49–55].

A review of 200 *Lactobacillus* infections found that 9% of the infections occurred in transplant recipients [54]. Patel et al. described *Lactobacillus* bloodstream infections in 8 patients within the first 6 months after liver transplantation [51]. All of the infections but one were polymicrobial, and in most cases the organisms were also isolated from abscess fluid or bile. The presence of a Roux-en-Y choledochojejunostomy was a strong risk factor for infection. Other reports of serious infection include endocarditis and mediastinitis in a heart recipient [49], splenic abscess in a kidney recipient with concomitant HIV infection [53] and relapsing bacteremia and meningitis in a cord blood transplant recipient [56]. A case of *Lactobacillus* pneumonia and empyema occurring early after lung transplantation was thought to be transmitted by the transplanted lung [52].

The current use of probiotics containing "non-pathogenic" microorganisms for various gastrointestinal conditions has raised concerns that this may be an unsafe practice in transplant patients. Indeed, serious *Lactobacillus* infections have been described in transplant recipients receiving probiotics that contained *Lactobacillus species* [57, 58]. In one case, an isolate from an empyema collection was found to be identical by molecular typing to the organism in the patient's probiotic preparation [58].

The standard treatment for *Lactobacillus* infection is high-dose penicillin or ampicillin, with or without an aminoglycoside for synergy [54, 55]. Other active antibiotics include erythromycin and other macrolides, carbapenems, linezolid, and quinupristin-dalfopristin [54, 55, 59]. Trimethoprim-sulfamethoxazole, metronidazole, and vancomycin have unreliable activity [51, 54, 55, 59]. Whenever possible, in vitro susceptibility tests should be obtained to direct therapy.

23.2.4 *Rhodococcus equi*

R. equi is a gram-positive coccobacillus of the order Actinomycetales. It is a veterinary pathogen that causes chronic suppurative pneumonia in foals and submaxillary lymphadenitis in swine [60]. Herbivores, such as horses and cattle, are colonized in the gut, and the organism inhabits soil contaminated by their manure. Approximately one-third of individuals with *Rhodococcus* infection report contact with farms or livestock [60]. *R. equi* infections occur primarily in patients who have defects in cell-mediated immunity. Approximately 10% of human *R. equi* infections occur in SOT and HCT recipients [61–64].

The lung is most common site of *Rhodococcus* infection. Patients present with a subacute course, characterized by fever, dyspnea, and nonproductive cough [60, 64, 65]. Other common symptoms include fatigue, weight loss, pleuritic chest pain and hemoptysis. Chest imaging demonstrates infiltrates or nodules, which frequently cavitate. Pleural effusions are common and often infected. Infection frequently disseminates to extrapulmonary sites such as the skin, bones, and brain. Disseminated infection is seen in roughly one-half of transplant recipients [61, 63].

The diagnosis of *Rhodococcus* requires laboratory isolation of the organism from a patient with a compatible clinical presentation. Early growth of *R. equi* may occur within 24–48 h, but the characteristic, mucoid, salmon-colored appearance of the colonies is not evident until a few days later [60]. The organism is easily missed in respiratory cultures and can be mistaken for "diptheroids" [66]; therefore, the laboratory should be alerted whenever *R. equi* infection is being considered. Blood cultures are positive in more than one-half of immunocompromised hosts [61, 65].

Many antibiotics are active against *Rhodococcus*. The most potent agents are vancomycin, imipenem, rifampin, quinolones, macrolides, and linezolid [61, 67, 68]. Clinical experience with linezolid is limited, but Munoz et al.

reported successful treatment of multidrug-resistant, relapsing *Rhodococcus* pulmonary infection in a heart transplant recipient [70]. Treatment with penicillins and cephalosporins has been unreliable and these antibiotics should be avoided. Treatment for immunocompromised hosts should consist of combination therapy with 2 or 3 active antibiotics. Intravenous therapy is commonly used initially, especially in patients with bacteremia or pulmonary abscesses. After the patient stabilizes and susceptibility results are available, therapy can be switched to an oral regimen. A treatment course of several months is typically required and it may be extended to 6 months or longer in disseminated infection [61]. Even with prolonged treatment, relapses may occur [71]. Adjunctive surgical therapy may be useful in selected patients [72].

23.2.5 *Clostridium difficile*

C. difficile is a spore-forming, gram-positive, anaerobic bacillus that elaborates toxins that cause colitis. Infection is usually associated with current or recent antibiotic use. *C. difficile* is part of the intestinal flora in approximately 3% of healthy adults, and as many as 30% of hospitalized patients [73]. Transmission in the health care setting is well documented.

The frequency of *C. difficile* infection is substantially higher in transplant populations than in other hospitalized patients. A meta-analysis, drawing on data from 21,683 SOT recipients, reported a pooled prevalence of *C. difficile* infection from transplantation to the first discharge of 7.4% [74]. The prevalence varied from 3.2% in pancreas transplant recipients to 10.8% in lung recipients. The *C. difficile* recurrence rate across the population was estimated to be 19.7%. In a large retrospective single center study spanning 6 years, the 1-year incidence of *C. difficile* infection among HCT recipients was 9.2%, with a breakdown of 6.5% among autologous and 12.5% among allogeneic recipients [75]. Relapsing *C. difficile* infection was observed in 21.7% of the patients at a median of 69 days after initial infection. Risk factor analysis showed the presence of gastrointestinal graft-versus-host disease (GVHD) to be highly associated with both overall and recurring *C. difficile* infection. *C. difficile* infection also correlated with the subsequent development of gastrointestinal GVHD. It was postulated that *C. difficile* infection triggered gastrointestinal GVHD by disruption of the mucosal barrier and release of proinflammatory cytokines. This association between *C. difficile* infection and gastrointestinal GVHD has been found in some but not all studies [76–78].

The symptoms of *C. difficile* infection in transplant recipients resemble those in other patients—watery diarrhea, lower abdominal pain and, at times, fever—and may be similar to symptoms of GVHD. The severity of *C. difficile* infection in transplant populations has been variably reported to be greater or less than in control populations [75–77, 79]. Authors who have found less severe disease in transplant recipients have speculated that immunosuppression attenuated the colonic inflammatory response and led to a less severe clinical course. It is also possible that less severe manifestations in transplant recipients were simply due to earlier diagnosis and treatment.

The cytotoxicity cell assay is the gold standard for diagnosis of *C. difficile* infection, but it is labor intensive and not widely used. Until recently the diagnosis was usually made by enzyme linked immunoassay (ELISA) for *C. difficile* toxin. Detection of *C. difficile* DNA by polymerase chain reaction (PCR) testing is more sensitive than ELISA and it is increasingly the primary test used for diagnosis. PCR testing has the drawback that it also detects asymptomatic carriage. Another accepted option for diagnosis is to employ a two-step algorithm, with an initial stool screen for a cell wall protein (glutamate dehydrogenase) common to both toxigenic and nontoxigenic strains, with subsequent testing by ELISA and/or PCR [80, 81].

The first consideration in treating *C. difficile* infection is cessation of the inciting antimicrobial agent(s) or transition to a narrower spectrum regimen, whenever possible. Management protocols for *C. difficile* infection generally recommend a stratified approach: oral metronidazole (500 mg every 8 h) is given for initial episodes of mild-to-moderate infection; oral vancomycin (125 mg every 6 h) is used for severe infection; and high-dose vancomycin (500 mg every 6 h) is administered orally or per rectum with or without intravenous metronidazole for severe, complicated infections [82]. Mild first recurrences can be retreated with oral metronidazole, but additional or severe recurrences should be managed with oral vancomycin using a tapered or pulse regimen. Fidaxomicin is an effective but costly alternative treatment which may be associated with a lower recurrence rate [83, 84]. For severely ill patients, especially those with toxic megacolon, colectomy may be a life-saving intervention.

No controlled studies are available to inform the treatment of *C. difficile* infection in transplant patients. Initial therapy is typically administered for 10–14 days, or longer if other antimicrobial therapy cannot be discontinued. If a prolonged duration of *C. difficile* treatment is planned, oral vancomycin is often the preferred agent to avoid the neurologic and hematologic toxicities associated with the extended use of metronidazole. Recently, fecal microbiota transplant (FMT) has emerged as an effective treatment for recurrent *C. difficile* infection [85]. Initial data on use of FMT in transplant recipients is limited but encouraging [86, 87]. There is a lack of conclusive data to support the use of probiotics to prevent *C. difficile* infection, particularly in transplant recipients where there is risk for bloodstream infection [57, 58, 82]. Prevention of *C. difficile* infection in populations at risk requires a multifaceted program, including aggressive infection control measures and an effective antibiotic stewardship program.

23.3 Gram-Negative Organisms

23.3.1 Legionella

Legionella are fastidious, aerobic, gram-negative rods that have been found in soil and freshwater lakes and streams. Over 50 species and 70 serogroups of *Legionella* have been described, and 20 species have been linked to human infection. The predominant species, *Legionella pneumophila* causes 95% or more of human illness in Europe and the USA [88]. Serogroup 1 makes up 80–90% of infectious isolates of *L. pneumophila*. Other *Legionella* species known to cause clinical infection in transplant patients include *Legionella micdadei, Legionella longbeachae*, and *Legionella dumoffii* [89–91].

Defects in cell-mediated immunity make transplant recipients particularly susceptible to legionellosis. Infection has been frequently reported in recipients of kidney [89, 90, 92], heart [93], liver [94], and hematopoietic cell [95, 96] transplants. *Legionella* infections can occur at any time after transplantation, but the frequency is greatest early after transplantation or following anti-rejection treatment.

Legionella infection is acquired by inhalation of infectious aerosols or by aspiration of infected water. Person-to-person transmission does not occur. Infections may be sporadic or part of health care-associated or community-associated outbreaks. Several outbreaks of *Legionella* infection in transplant recipients have been described [97–99]. *Legionellae* have a major, clinically relevant reservoir in institutional plumbing systems. They enter these systems via cold water intakes and subsequently colonize hot water heaters, from which they are dispersed to spigots and showerheads on patient wards [100, 101]. Outbreaks of *Legionella* pneumonia have been epidemiologically linked to several sources, including potable tap water, cooling towers, evaporative condensers, humidifiers, whirlpools, and decorative water fountains [88, 100, 102–107].

Pneumonia is the usual clinical presentation of *Legionella* infection. Some clinical features may suggest the diagnosis of legionellosis. Patients often experience a flu-like prodrome of high fever, chills, myalgias, and malaise, but antecedent upper respiratory symptoms are usually absent. Progressive infection results in dyspnea and a mildly productive cough, which is often associated with pleuritic chest pain. Approximately one-half of patients develop watery diarrhea. Mild CNS symptoms, such as headache and confusion, are often present. Some investigators have noted the presence of a pulse–temperature disassociation with a relative bradycardia [92]. The most common radiographic appearance of *Legionella* pneumonia is alveolar consolidation, which is frequently multilobar [108]. Pleural effusions, cavitation may be seen and focal nodular densities, mimicking invasive fungal infection, have been described [109] (see Figure 23-3).

Extrapulmonary *Legionella* infection is a rare occurrence, usually seen in immunocompromised hosts, with or without primary pneumonia. Some reported types of extrapulmonary involvement are cutaneous infection [110], aortitis [111], prosthetic valve endocarditis and sternal wound infection [112]. The morbidity of legionellosis in transplant recipients is substantial; the reported mortality ranges from 14 to 30% [96, 113], but can be as high as 80% for untreated health care-associated infection [92].

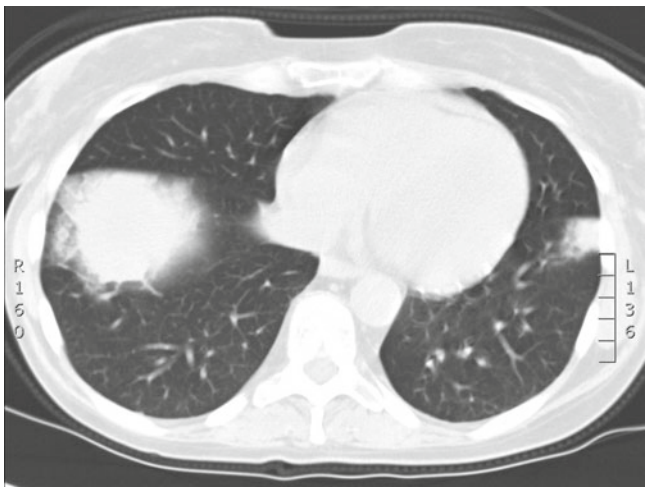

FIGURE 23-3. CT chest demonstrating multifocal nodular consolidations in a stem cell transplant recipient on high-dose immune suppression, demonstrated to have *Legionella* infection.

The laboratory diagnosis of legionellosis is often difficult and depends on the available level of expertise. Although *Legionella* are gram-negative bacilli, they are usually not visualized on Gram stain because of their small size and poor stain avidity. The definitive method of diagnosis is by culture. *Legionella* are fastidious and their isolation requires the use of enriched media (buffered charcoal yeast extract agar) in a CO2-rich environment (see Figure 23-4). Colonies appear after 3–5 days on agar plates but may be masked by overgrowth of other less fastidious organisms. Cultured organisms are more readily visualized by Gram stain than those in clinical specimens. It is notable that on tissue biopsy specimens *L. micdadei* can demonstrate weak acid-fast staining, highlighting the importance of *Legionella* culture when this diagnosis is suspected [114].

Several indirect methods for *Legionella* diagnosis are available. Direct fluorescent antibody (DFA) staining of sputum or tissue specimens is a rapid technique, but it has a low sensitivity (50%), and reagents are lacking for some species and serogroups [115]. *Legionella* serology has been useful for epidemiological studies but has limited value for diagnosis. The detection of urinary antigen is a well-established, rapid-turnaround assay with a sensitivity of greater than 85% for infections caused by *L. pneumophila* serogroup 1, but has little utility for the diagnosis of infection by other *Legionella* species. In the current era, most diagnoses of legionellosis are made by urinary antigen testing [88, 116]. Methods employing detection of *Legionella*

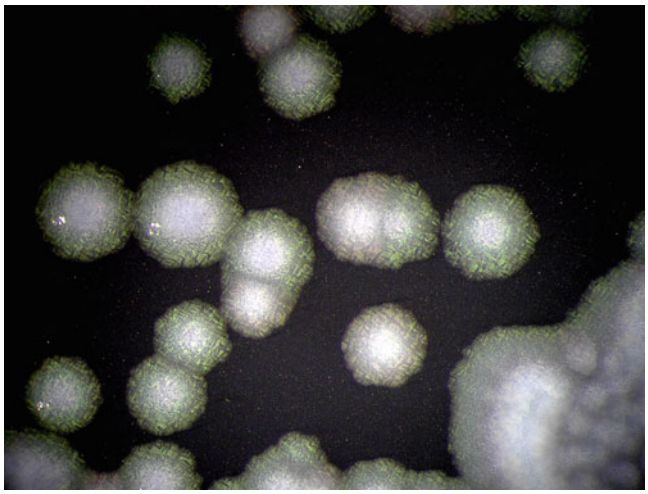

FIGURE 23-4. Colonies of *Legionella pneumophila* on buffered charcoal yeast extract (BCYE) agar. (Photo provided courtesy of A. William Pasculle Sc. D, Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA).

DNA by PCR have been developed and have potential for clinical application [88].

The most active antibiotics for *Legionella* treatment are the newer macrolides, such as azithromycin, and fluoroquinolones, especially levofloxacin and moxifloxacin. Erythromycin, rifampin, the tetracyclines, and TMP-SMX also have activity. All beta-lactam antibiotics, the aminoglycosides, vancomycin, and clindamycin are ineffective [92, 117–119]. Data regarding the benefit of combination therapy with rifampin are inconclusive [120], and drug-drug interactions pose a major drawback to the use of rifamycins in transplant populations.

Isolation for hospitalized patients with *Legionella* infection is unnecessary. If a hospital outbreak of legionellosis is detected, surveillance should include culturing of hospital water sources. Routine environmental sampling for *Legionella* in hospitals has been adopted in several states and by the US Veterans Affairs Healthcare System. It has also been recommended by the CDC for institutions with HCT programs. Community outbreaks of legionellosis have been linked to recreational or occupational exposure to aerosolized water, such as occurs in whirlpool spas or commercial water displays. It is therefore prudent to warn transplant recipients about the potential hazards of prolonged exposure to such aerosols.

23.3.2 Bartonella

Bartonella henselae is a fastidious, gram-negative bacillus that has a natural reservoir in domestic cats. Immunocompetent patients with *B. henselae* infection typically develop painful regional adenopathy and fever after a cat bite or scratch ("cat scratch disease"). Bacillary peliosis, bacillary angiomatosis, and persistent bacteremia with fever are typical manifestations of disseminated infection seen in patients with AIDS and other immunosuppressed hosts. Patients with bacillary peliosis have studding of the liver and spleen with numerous small inflammatory nodules that appear as hypodense lesions on computed tomography scanning. Bacillary angiomatosis is a vasculoproliferative form of disseminated *B. henselae* infection associated with lytic bone lesions and characteristic violaceous, friable skin nodules [121–123].

B. henselae infection has been reported in kidney, liver, heart, lung, and HCT recipients [121, 124]. Transplant recipients may present with localized cat scratch disease or with one or more of the syndromes associated with disseminated disease. Of 29 cases of *B. henselae* infection in SOT recipients reported by Psarros and coauthors, two-thirds were classified as disseminated [121]. An unusual form of bacillary angiomatosis with vegetating papillomatous lesions in the oral cavity has been described following HCT [124]. *Bartonella* endocarditis has been described in transplant recipients [121]. Unusual manifestations of *Bartonella* infection reported in kidney recipients include hemophagocytic syndrome [125] and vasculitis with allograft glomerulonephritis [126].

Donor-transmitted bartonellosis has been suspected in some cases, one of which had good documentation [121, 127]. In this case, a pediatric liver recipient was found to have a nodule of the liver and enlarged abdominal lymph nodes 2 months after transplantation [127]. Biopsies of the liver and lymph nodes showed granulomatous changes. *Bartonella* infection was confirmed by PCR of the liver. The recipient had no cat exposure and the donor was found to be seropositive for *B. henselae.*

Unlike *B. henselae*, humans are the only known reservoir of *Bartonella quintana. B. quintana* is transmitted by the human body louse, *Pediculus humanus*, and is the etiologic agent of trench fever. There is a single report of *B. quintana* infection after SOT presenting as bacillary angiomatosis in a kidney recipient from the Czech Republic [128].

Bartonella organisms are not routinely isolated from blood. Culture of tissue specimens on blood or chocolate agar may require an incubation period of 30 days and is not sensitive. For this reason, performing PCR on tissue specimens or blood is increasingly relied upon for diagnosis [129, 130]. Serology can be used as supportive evidence of infection in the appropriate clinical setting [121]. The diagnosis is strongly suggested when typical pathological changes are found in tissue sections, especially if Warthin–Starry stains of the tissue show organisms.

Cat scratch disease generally resolves without therapy in immunocompetent hosts. One small randomized trial showed a greater decrease in volume of affected lymph nodes in patients treated with azithromycin as compared with placebo [131]. No studies specifically address the treatment of bartonellosis in transplant recipients. Given the theoretical risk for dissemination, it seems prudent to recommend antimicrobial therapy for all transplant recipients with *Bartonella* infection.

Based on 2004 recommendations [132], a 5-day course of azithromycin is recommended as first-line treatment for cat scratch disease. Treatment for disseminated bartonellosis has not been studied, but observational data suggest that both macrolides and doxycycline should be effective agents; a treatment duration of 3–4 months is recommended [132]. Rifampin and gentamicin appear to be active agents and might be considered for adjunctive treatment of difficult or refractory cases [132]. Prevention of bartonellosis in transplant populations entails counseling individuals to avoid contact with cats, particularly younger cats, as they are associated with the highest risk for transmission [133].

23.3.3 *Bordetella bronchiseptica*

Bordetella bronchiseptica is a small, pleomorphic, aerobic, gram-negative coccobacillus. It is a cause of infection in household and farm animals and is known among pet owners as the etiologic agent of "kennel cough." Most human infections with *B. bronchiseptica* occur in immunocompromised hosts. There are numerous reports of *B. bronchiseptica* infection after SOT and HCT [134–140]. Most patients present with fever and cough. Findings on chest imaging are variable and include infiltrates, nodular densities, or cavities [136]. Some patients develop respiratory failure or bacteremia. The organism can be cultured from respiratory secretions or blood using standard laboratory techniques.

Infected patients frequently report contact with animals and sometimes there is a documented or suspected infection in a household pet [135, 137, 138]. In one instance, a kidney–pancreas transplant recipient with pneumonia appeared to have acquired the organism from a pet dog that had been immunized with live-attenuated, intranasal *B. bronchiseptica* vaccine [134]. In another report, two allogeneic HCT recipients at the same center developed severe *B. bronchiseptica* infection within 3 days of each other [139]. Pulsed-field gel electrophoresis analysis indicated that the two patients' isolates were identical. Neither patient reported contact with animals after transplantation, but both were being treated in the same transplant ward and clinic, suggesting the possibility that *B. bronchiseptica* was transmitted in the health care setting.

There are no definitive guidelines on treatment of *B. bronchiseptica* infection. The organism is often susceptible to erythromycin and azithromycin, antipseudomonal penicillins, third-generation cephalosporins, TMP-SMX, aminoglycosides, tetracyclines, and fluoroquinolones [135, 136, 139]; it is usually resistant to penicillin, ampicillin, and clindamycin [135–137]. Treatment is complicated by the fact that antibiotic susceptibilities do not always predict the clinical response. Patients may suffer microbiological or clinical relapses, possibly due to the capacity of *B. bronchiseptica* to invade and persist in respiratory epithelium and alveolar macrophages. Emergence of resistance after antibiotic treatment has also been documented [134, 137, 140]. Infection with *B. bronchiseptica* might be prevented by advising transplant recipients to avoid close contact with animals that are sick or have recently received live *B. bronchiseptica* vaccine.

23.3.4 *Helicobacter pylori*

H. pylori is a curved, gram-negative bacillus that infects 25–50% of adults in developed countries and causes chronic gastritis [141]. Infection with *H. pylori* has been definitively linked to the occurrence of peptic ulcer disease, and it is a major risk factor for the development of gastric cancer. Infected persons develop antibodies to *H. pylori*, and seropositivity is a reliable indicator of chronic infection in the stomach.

Several studies have investigated *H. pylori* infection in transplant recipients. An investigation found that 29% of 202 kidney transplant recipients were seropositive for *H. pylori* antibodies, a rate similar to patients on hemodialysis [142]. Seropositivity was associated with dyspeptic symptoms. In another study, 48% of 33 kidney recipients undergoing upper endoscopy between 2 and 4 months after transplantation had *H. pylori* identified by histology or urease testing [143]. The *Helicobacter*-infected patients were more likely to have gastritis, peptic ulcers, or dyspeptic symptoms. Somewhat disparate results were seen in a longitudinal study of 100 heart transplant recipients, 35% of whom were seropositive before transplantation [144]. Only 1 of the 65 seronegative patients seroconverted over a follow-up of 3.5 years. Seropositive patients did not have more episodes of ulcer disease, gastritis, or gastrointestinal bleeding than seronegative patients, but 40% of *Helicobacter*-seropositive patients became seronegative in follow-up. This finding correlated with a more intensive use of antibiotics, which appeared to have inadvertently cured the patients of their *Helicobacter* infections. Similar reversions to seronegative status have been reported from liver transplant recipients in Germany [145] and kidney transplant recipients in Finland [146].

Information on *Helicobacter* infection in patients undergoing HCT is limited. A study of 276 HCT recipients undergoing endoscopy, either before or after transplantation, disclosed only one case of *H. pylori* infection [147]. Castagnola et al. diagnosed *H. pylori* infection using a stool antigen assay in 13 (3%) of 478 children with hematologic malignancy, including 3 children who had undergone HCT [148]. Patients presented with dyspepsia or gastrointestinal bleeding and all improved with treatment of the *Helicobacter* infection; however, there was no direct evidence that *Helicobacter* infection had caused the patients' symptoms.

Despite limitations, these studies suggest that SOT and HCT recipients are not more likely, and they may be less likely, to be chronically infected with *H. pylori* than the general population. The available data does not answer the question whether transplant recipients with *Helicobacter* infection are more or less likely than immunocompetent hosts to develop ulcer disease.

An intriguing manifestation of *H. pylori* infection in transplant recipients is the occurrence of mucosa-associated lymphoid tissue (MALT) B-cell lymphomas. MALT lymphomas in the stomach are associated with *H. pylori* infection. They have been reported to respond to and even be cured by treatment of *Helicobacter* infection, thus obviating the need for cancer chemotherapy. Four cases of gastric MALT lymphoma were described in 1850 liver transplant recipients, a rate of 0.2%, which is 10–100 times more common than in the general population [149]. MALT lymphomas have also been described in heart and kidney recipients [150]. The mortality of MALT lymphomas appears low compared to other transplant tumors, as only two deaths from malignancy were seen among 16 cases in a transplant tumor registry [150].

The most commonly used regimen for *H. pylori* infection is 3-drug therapy, consisting of a proton pump inhibitor, clarithromycin, and amoxicillin [141]. Metronidazole is substituted for amoxicillin in penicillin-allergic patients. In areas where clarithromycin resistance is high, a 4-drug regimen is preferred. The most common 4-drug regimen employs a proton pump inhibitor, bismuth subsalicylate, metronidazole, and a tetracycline compound. Confirmation of cure is recommended when patients have persistent symptoms, underlying ulcer disease or a *Helicobacter*-associated cancer. Testing can be done with a urea breath test, a stool antigen assay, or repeat endoscopy and should be delayed until at least 4 weeks after the end of therapy [141].

23.4 Mycoplasma

23.4.1 *Mycoplasma* and *Ureaplasma*

The mycoplasmas and ureaplasmas differ from most bacteria in their small size (150–250 nm) and lack of a cell wall. The principal species causing transplant infections are *Mycoplasma pneumoniae*, which is a respiratory pathogen, and *M. hominis* and *Ureaplasma urealyticum*, which have a role in minor genitourinary infections, but occasionally cause severe extragenital disease [151, 152]. Because these organisms cannot be detected on Gram stain and their culture requires specialized techniques, only a minority of clinical infections receive an etiologic diagnosis.

M. pneumoniae is a common cause of bronchitis and pneumonia [151], but it is also is associated with a number of interesting extrapulmonary conditions, including cold agglutinin-positive hemolysis and Stevens–Johnson syndrome. Occasional patients with *M. pneumoniae* infection develop secondary carditis or CNS diseases such as aseptic meningitis or encephalitis [153, 154]. An unusual case of disseminated *M. pneumoniae* infection was diagnosed in a kidney recipient by PCR testing from multiple infected sites, including an axillo-femoral bypass graft, the knee joint, and a psoas abscess [155]. *M. pneumoniae* with Stevens–Johnson syndrome has been described in HCT and liver transplant recipients [156, 157]. Chronic *M. pneumoniae* pulmonary infection was reported in a pediatric kidney transplant recipient with hypogammaglobulinemia, The patient had fever and respiratory symptoms for 6 months and the diagnosis was finally established by PCR testing of BAL fluid [158].

M. hominis and *Ureaplasma species* have been isolated from the genitourinary flora of many sexually experienced men and women. They are often commensals, but seem to play a role in some common infections, such as nongonococcal urethritis (*U. urealyticum*) and bacterial vaginosis (*M. hominis*) [152]. *M. hominis* infections outside the genitourinary tract are well described, particularly in patients who are postpartum or have compromised immune systems [159]. Two-thirds of 17 patients in a case series of invasive extragenital *M. hominis* infections were immunosuppressed [159]. *M. hominis* infection has been reported in all types of SOT recipients [159–163]. Mixed infection with *M. hominis* and *U. urealyticum* has been reported in kidney, liver, and lung recipients [164–166].

Superficial or deep *M. hominis* sternal wound infections in heart and heart–lung recipients usually occur within a few weeks of transplantation. These patients have fever with sternal inflammation and drainage but Gram stains and cultures of the drainage are negative [167, 168]. Some reports of early posttransplant *M. hominis* pneumonia in lung recipients are suspicious for donor transmission, including a case of *M. hominis* and *U. urealyticum* coinfection [160, 166]. Cases of *M. hominis* deep wound infection reported following kidney transplantation seem likely to be due to spread of *Mycoplasma* colonizing either the donor or recipient urinary tract [169]. Other well-documented types of *M. hominis* infection are septic arthritis, peritonitis, meningitis and bacteremia [159]. *M. hominis* infection is rare after HCT. In the only reported case, *M. hominis* was cultured from BAL fluid, pharyngeal secretions and urine of a patient with diffuse alveolar hemorrhage [170]. It was unclear if the organism had any causal role in the clinical illness.

Diagnosis of *Mycoplasma* infection requires a high index of suspicion, as routine stains of purulent material are negative. Translucent colonies may be seen after 4–5 days of culture on blood agar plates but are often mistaken for water droplets. The organism grows best on *Mycoplasma*-specific media, producing colonies with a "fried egg" appearance. Because of these diagnostic challenges, clinicians should alert the microbiologist if *Mycoplasma* infection is suspected. Increasingly, diagnosis is made by PCR [155, 162].

Macrolides, tetracyclines and fluoroquinolones are active against *M. pneumoniae*, but resistance to macrolides has recently emerged in Asia and will undoubtedly spread to other countries [151, 171]. Most *M. hominis* isolates are sensitive to clindamycin, rifampin, fluoroquinolones, and tetracycline [159]. The organism is resistant to other macrolides, aminoglycosides, sulfonamides, and cell wall-active agents

including beta-lactam antibiotics. The treatment duration should be at least 2 weeks or longer when severe, deep-seated infection is present. There is a paucity of laboratory data to guide treatment of *Ureaplasma* infection, but resistance to macrolides and tetracycline appears to be increasing. Fluoroquinolones, particularly moxifloxacin, should be considered for management of complicated or refractory cases [172, 173].

23.5 Spirochetes

23.5.1 *Treponema pallidum*

T. pallidum, the causative organism of syphilis, is a non-cultivatable spirochete. The primary modes of acquisition are sexual contact, transplacental passage to the fetus or, rarely, accidental direct inoculation. Infection can also be transmitted by blood transfusion, but the risk is low because blood donors are screened and the organism cannot survive for longer than 24–48 h in stored blood units [174].

The potential for transmission of syphilis by donated organs is a concern and organ donors are routinely screened for syphilis. Transplantation of organs from donors with positive syphilis serology has been reported in kidney, liver, and lung transplantation [175, 176]. In these cases, the donors had no symptoms of active syphilis and were thought to have latent or convalesced infection. Transient seroconversion of both treponemal and non-treponemal serology occurred in some recipients, but without evidence of acute clinical disease or sequelae [175–177]. All recipients received penicillin therapy posttransplantation. Serological conversion may have represented an immune response to asymptomatic infection; however, it was also proposed that serological conversion in the recipients may have derived from antibodies elaborated by donor-derived B cells. This was likely the case in one Japanese patient who seroconverted their syphilis serology after allogeneic HCT [178]. The sibling donor had positive syphilis serology but had been appropriately treated with penicillin before harvest of stem cells. The available data are limited but suggest that screening of SOT and HCT donors should continue. Administering high-dose penicillin to recipients posttransplantation or to living donors pretransplantation should be adequate to prevent significant disease.

There are reports of active secondary syphilis in SOT recipients [179–181]. The clinical findings were typical and included fever, diffuse skin rash (see Figure 23-5), hepatitis and various neurological symptoms such as headache, dysesthesias and visual changes. Neurosyphilis was documented in over one-half of the patients. All patients responded well to antibiotic treatment.

The diagnosis of syphilis is based on serologic testing for non-treponemal antibodies (VDRL test or rapid plasma reagin) accompanied by a specific treponemal antibody test such as *T. pallidum* particle agglutination [TP-PA]. The diagnosis can also be suggested when typical pathological changes are found on tissue biopsy of affected organs [179, 181]. The treatment of choice is parenteral penicillin. Doxycycline or erythromycin are second-line agents for patients with penicillin allergy.

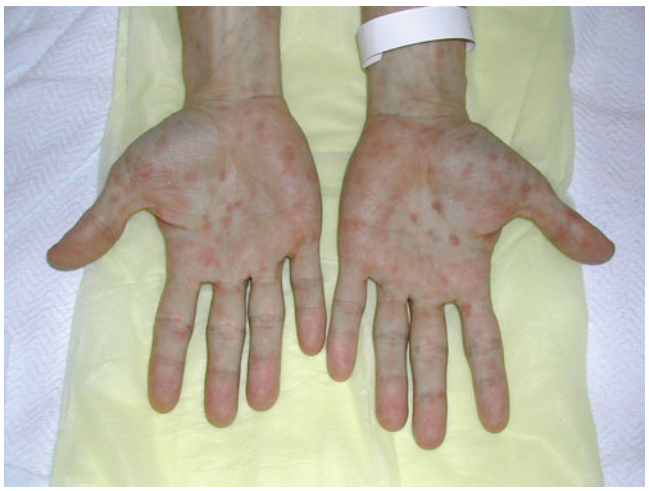

FIGURE 23-5. Classic skin findings of secondary syphilis. (Photo provided courtesy of Kent Sepkowitz, M.D., Division of Infectious Diseases, Memorial Sloan Kettering Cancer Center, New York, NY).

23.5.2 Borrelia

Lyme disease is an infection transmitted by *Ixodes* ticks and caused by various *Borrelia* species (*B. burgdorferi* and *B. pacificus* in the USA, and primarily *B. afzelii* and *B. garinii* in Europe and Asia). The animal reservoirs in the USA are small rodents, but large animals, such as deer and cattle, support the life cycle of the *Ixodes* ticks. Lyme disease has been reported in SOT recipients [182–184] and in a HCT recipient [185], with manifestations ranging from localized erythema migrans to disseminated disease with carditis or neurologic involvement. Given the paucity of data on Lyme disease in transplant recipients, it is not clear if the severity of disease is greater than in immunocompetent patients.

Because serologic diagnosis of infection has potential limitations in immunocompromised patients, a high index of suspicion is warranted in any transplant patient who has exposure in an endemic area and has signs or symptoms suggestive of Lyme disease [184, 185]. Treatment is universally indicated in patients with active Lyme disease. Oral doxycycline is commonly prescribed for most patients with erythema migrans, those with isolated facial nerve palsy or with arthritis without neurologic involvement. Parenteral therapy (e.g., ceftriaxone) is usually reserved for patients who have disseminated disease with neurologic or cardiac involvement [186].

23.6 Rickettsiosis

23.6.1 *Coxiella burnetii*

C. burnetii, the etiologic agent of Q fever, is a pleomorphic gram-negative coccobacillus. Infection usually arises from exposure to infected livestock or unpasteurized milk. The most common clinical manifestation is an acute febrile illness associated with pneumonia or hepatitis. Many patients who are untreated recover, but a small proportion may develop a chronic febrile illness, often accompanied by endocarditis.

C. burnetii infection has been reported in patients with a variety of immunocompromising conditions, [187], and there are a few reports of acute or chronic Q fever after SOT and HCT that have demonstrated response to doxycycline-containing regimens [188–191]. Although the literature on Q fever in the transplant population is sparse, treating a patient who has a positive serologic response seems prudent to prevent chronic infection, even when symptoms are mild or resolving.

23.6.2 Other Rickettsial Organisms

Rickettsia, *Ehrlichia*, and *Anaplasma* species are members of the order *Rickettsiales*. They are fastidious, obligate intracellular bacteria with gram-negative cell walls, but are poorly visualized by Gram stain. A number of these organisms are important human pathogens that are transmitted by arthropod vectors.

A single case report describes a heart transplant recipient with Rocky Mountain spotted fever, a tick-borne illness caused by *Rickettsia rickettsi* [192]. The patient had a mild febrile illness with rash and responded to 3 weeks of treatment with doxycycline. The diagnosis was initially made by immunofluorescence staining of a skin biopsy and later confirmed by seroconversion. There are two case reports of SOT recipients with Mediterranean spotted fever, a tick-borne illness caused by *Rickettsia conorii.* One kidney recipient presented with rash and spontaneous splenic rupture after recent travel to southern France; the patient improved on empiric doxycycline and the diagnosis was ultimately made by PCR amplification of spleen and skin tissue [193]. The second patient was a liver recipient in Spain who developed high fever, myalgias and rash; the diagnosis of *R. conorii* infection was made serologically and the patient responded promptly to doxycycline treatment [194]. Murine typhus is caused by *Rickettsia typhi* and transmitted by fleas. A single case of murine typhus was reported in a liver transplant recipient from Thailand who had fever, hepatitis, and interstitial pneumonia; the diagnosis was made by serology and treatment with doxycycline resulted in clinical cure [195]. Murine typhus may be encountered around the world and some recent cases have been described in Texas and Southern California [196].

Human ehrlichiosis and anaplasmosis have similar clinical manifestations but differ in their geographic distributions, the tick vector and the specific blood cells—either monocytes or granulocytes—that support infection. Human monocytic ehrlichiosis is caused by *Ehrlichia chaffeensis* and transmitted by the lone star tick (*Amblyomma americanum*). It was first reported in a liver transplant recipient from Kentucky in 1995 [197]. This patient developed fever, pancytopenia, elevated transaminases, and shortness of breath 2 weeks after a tick bite. He made a full recovery on empiric doxycycline therapy, with the diagnosis subsequently established by serology. Human granulocytic anaplasmosis is caused by *Anaplasma phagocytophilum* and transmitted by *Ixodes scapularis*. It was first reported in a kidney transplant recipient from Minnesota who developed fever, myalgia, diarrhea, and pancytopenia a week after tick exposure [198]. *Ehrlichia ewingii* is the agent of canine granulocytic ehrlichiosis, It was first reported to cause human infection in 1999 [199]. Infection with *E. ewingii* generally produces a milder illness than *E. chaffeensis*. Most reported cases have been in immunocompromised hosts, including transplant recipients [199, 200].

Cellular immunity is an important host defense against rickettsial infection and poor outcomes have been reported in HIV-seropositive individuals [201, 202]. It is not clear if transplant recipients have more severe disease or worse outcomes. Thomas et al. compared clinical characteristics of ehrlichiosis (both *E. chaffeensis* and *E. ewingii*) in 15 SOT patients and 43 immunocompetent patients [200]. Transplant recipients had less rash and less hepatic enzyme elevation but more leukopenia and renal dysfunction than the immunocompetent patients. All transplant patients responded rapidly to doxycycline therapy and their mean hospital stay was only 4 days. In a review of 23 immunocompromised patients with *Ehrlichia* infection, severe disease occurred in some of the 7 SOT recipients, but they all survived; the six deaths reported in the series occurred in patients with HIV or splenectomy [202].

A case of probable donor-derived *Ehrlichia* infection has been reported in two kidney recipients of a common donor [203]. Both recipients developed high grade fever in the early posttransplant period. The diagnosis was made by detection of *E. chaffeensis* DNA by PCR from serum in one recipient and by serology in the other; unfortunately, no serum or tissue was available from the donor for confirmatory testing.

Monocytic ehrlichiosis and anaplasmosis can sometimes be diagnosed by finding "morulae," or characteristic intracytoplasmic inclusions, in a buffy coat smear. Morulae are found in monocytes in monocytic ehrlichiosis but are uncommon (<10%); they are found in granulocytes in anaplasmosis and are relatively frequent (20–80%). Serology for *E. chaffeensis* and *A. phagocytophilum* is useful for retrospective diagnosis and epidemiologic studies, but not rapid enough for clinical purposes. Specific serology is not available for *E. ewingii*, although patients with *E. ewingii* infection

often develop cross-reactive antibodies to *E. chaffeensis* [199]. Culture is not commercially available. PCR testing is now the preferred way to make a rapid, species-specific diagnosis [199, 200, 204]. Empirical treatment should be initiated in patients with suspected ehrlichiosis or anaplasmosis, pending results of diagnostic testing. The agent of choice is doxycycline. In regions endemic for anaplasmosis doxycycline will treat for the possibility of coinfection with *B. burgdorferi*, which is also transmitted by *Ixodes* ticks.

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Part V
Viral Infections

24
Cytomegalovirus Infection After Stem Cell Transplantation

Morgan Hakki and Per Ljungman

24.1 Virus Structure and Replication

Human cytomegalovirus (CMV) is a member of the beta (β) herpesvirus subfamily, along with human herpesvirus (HHV)-6 and HHV-7. The CMV virion shares structural similarities with other herpesviridae. Namely, the double-stranded DNA genome is encased within an icosahedral capsid, which in turn is surrounded by a proteinaceous tegument (or matrix). A lipid membrane containing surface viral glycoproteins that function in host cell binding and entry is the outermost component of the virion.

The CMV genome is approximately 230 kb, making CMV one of the largest among human viruses, and is organized into unique long (UL) and unique short (US) segments that are flanked by inverted genomic repeats. Most CMV genes are named according to their position within the genome based on the reference strain AD169 [1]. For example, UL97 is the 97th open reading frame (ORF) in the UL segment and US28 is the 28th ORF in the US segment. CMV genes may also have names that reflect historical usage, function, or homology to genes of other herpesviruses.

Like all herpesviruses, CMV establishes latency after primary infection, during which replication-competent virus remains present in the infected cell but evidence of viral replication is undetectable until triggered to reactivate. The viral and host factors that regulate latency and reactivation are poorly understood [2]. The site(s) of latency are not well defined but bone marrow stem cells of the myeloid lineage such as CD34+ and CD14+ cells have been shown to be one site of CMV latency [3, 4]. It has also been shown that the allogeneic effect can contribute to reactivation from peripheral blood mononuclear cells [5]. Since CMV can be transmitted from donor to recipient during solid organ transplant [6], parenchymal cells in these organs may also harbor latent virus.

24.2 CMV and the Host Immune System

24.2.1 Adaptive Immunity

Infection with CMV is associated with pronounced induction of $CD4^+$ and $CD8^+$ T cell responses. Immunodominant T cells responses are directed primarily against the gene products of UL123 (IE-1) and UL83 (pp65) [7–12]. However, CMV-specific T-cell immunity is now recognized as complex due to the large numbers of antigens, both lytic and latency-associated, that have been found to be targeted by T-cell responses [13–16]. Numerous studies have documented the importance of both CMV-specific $CD8^+$ and $CD4^+$ responses in determining the incidence and outcome CMV infection after allogeneic HCT [17–26]. Similar findings have been observed after newer HCT techniques such as haploidentical HCT [27] and umbilical cord blood transplant (CBT) [28–30].

The contribution of humoral immunity in controlling CMV replication is less clear. Antibodies to glycoprotein B (gB) and glycoprotein H (gH) predominate during infection [31–33], but while such antibodies may neutralize virus in tissue culture, their capacity to prevent primary infection is not well defined. While evidence suggests that antibody may serve to limit CMV dissemination and disease severity [34–36], lack of antibody does not alter the course of the primary MCMV infection in murine models [36]. Thus, the contribution of antibody to the control of CMV infection remains poorly understood.

24.2.2 Innate Immunity

Innate immunity plays a critical role in controlling herpesvirus infections through the production of inflammatory cytokines such as type I interferons (IFN α and β), interleukin 12

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(IL-12), and tumor necrosis factor (TNF) that exert a direct antiviral effect and induce adaptive immunity [37, 38]. The CMV glycoproteins gB and gH trigger the toll-like receptor 2 (TLR2) upon binding to the target cell [39–41]. In addition, viral DNA triggers TLR3 and TLR9 as well as the DNA sensor ZBP1 [42–47]. Attempts to correlate polymorphisms in donor and recipient TLRs and other innate immune sensors with CMV infection after HCT have yielded conflicting results that require further study [48–52].

Expansion of natural killer (NK) cells during CMV infection has been reported in both immunocompetent humans and after HCT [53–58]. While NK cells have been shown to limit MCMV replication in mice [59–63], their role in controlling CMV infection in humans is less clear although associative evidence strongly indicates an important contribution [22, 64, 65]. In addition, the genotype of the donor activating KIR (aKIR) has been demonstrated to influence the development of CMV infection after allogeneic HCT [66–68]. The mechanistic basis underlying these correlative findings is not well defined.

γδ T cells represent a minority (<6%) subset of circulating T cells in healthy individuals but are more prominent in peripheral sites such as mucosal surfaces [69]. Marked by the expression of receptors composed of γ and δ chains [38], as opposed to α and β chains associated with CD4+ and CD8+ responses, they respond to CMV infection with both in both innate- and adaptive- type immune function [70, 71]. CMV infection stimulates γδ T cell proliferation in both humans and mice, and deficient γδ T cell function has been associated with impaired regulation of CMV infection [70, 72–74].

24.2.3 Immune Evasion Mechanisms

As a successful human pathogen, CMV has necessarily evolved numerous mechanisms to evade and counteract virtually all aspects of the host immune response. Starting at the earliest stages of infection, CMV utilizes virion-associated and immediate-early proteins to effectively prevent host cell apoptosis, interferon-mediated pathways, and other innate immune responses such as shutoff of host cell protein synthesis in response to viral nucleic acid accumulation [75–79]. Multiple CMV proteins as well as the noncoding viral microRNAs miR-UL122 and miR-112 inhibit NK cell function [80–82].

A hallmark of CMV immune evasion is the blunting of CTL responses by inhibiting MHC-I restricted antigen presentation [83]. A number of CMV proteins contribute to this, including the tegument protein pp65 and genes of the US2-11 region [84–92]

Finally, CMV encodes several homologues of cellular proteins, including MHC class-I molecules, chemokine receptors, IL-10, TNF receptors, and CXC-1 homologues, that function to evade the host immune response [93–97].

24.3 Diagnostic Methods

The serologic determination of IgG and IgM has an important role in determining a patient's risk for CMV infection after HCT (see below, "Risk Factors") but is not useful in the diagnosis of active CMV infection or disease.

Histopathologic examination of tissue specimens remains the "gold standard" in the diagnosis of invasive CMV disease. In addition to observing nonspecific viral cytopathic effect in tissue, immunohistochemical techniques are used to identify CMV antigens (Figure 24-1a left and middle panels).

Growth of CMV in tissue culture takes several weeks, limiting its clinical usefulness as a diagnostic tool. Culture-proven viremia is highly predictive of CMV disease, but is of limited utility for screening since this finding frequently coincides with the onset of symptomatic disease [98–100].

The shell vial technique, in which monoclonal antibodies are used to detect CMV immediate-early proteins in cultured cells, can be performed within 18–24 h after inoculation. This assay is not sensitive enough to use for routine blood monitoring [99], but is highly useful on bronchoalveolar lavage (BAL) fluid in the diagnosis of CMV pneumonia due to its established specificity in this setting [101]. Many laboratories have abandoned culture-based techniques in favor of nucleic acid testing so that today these techniques have limited availability in many parts of the world.

The detection of the CMV pp65 tegument phosphoprotein in peripheral blood leukocytes offers a rapid, sensitive, and specific method of diagnosing and roughly quantitating CMV viremia. In the transplant setting, a positive or quantitatively increasing CMV pp65 assay has been shown to predict the development of invasive disease [102, 103] but is not always positive in the setting of proven end-organ disease, particularly gastrointestinal tract disease [104–107]. The predictive value of this assay has not been validated when performed on other body fluids such as BAL fluid. Since this assay relies on the detection of pp65 in circulating leukocytes, it may not be reliable in patients with profound leukopenia, such as in the pre-engraftment stage after HCT. At most centers, this assay has been replaced by nucleic acid testing primarily using quantitative polymerase chain reaction (qPCR).

qPCR relies on the amplification and quantitative measurement of CMV DNA. PCR is the most sensitive method for detecting CMV [108], while at the same time maintains high specificity. In addition, it is very rapid, with results usually available within 24 h, and does not rely on the presence of circulating leukocytes as does the pp65 antigenemia assay. qPCR provides a direct quantitative measurement of circulating CMV viral load, which is an accurate predictor of CMV disease after transplantation in most cases [109–113]. Like the pp65 antigenemia assay, serum or blood PCR may be negative in the setting of visceral disease [104, 106,

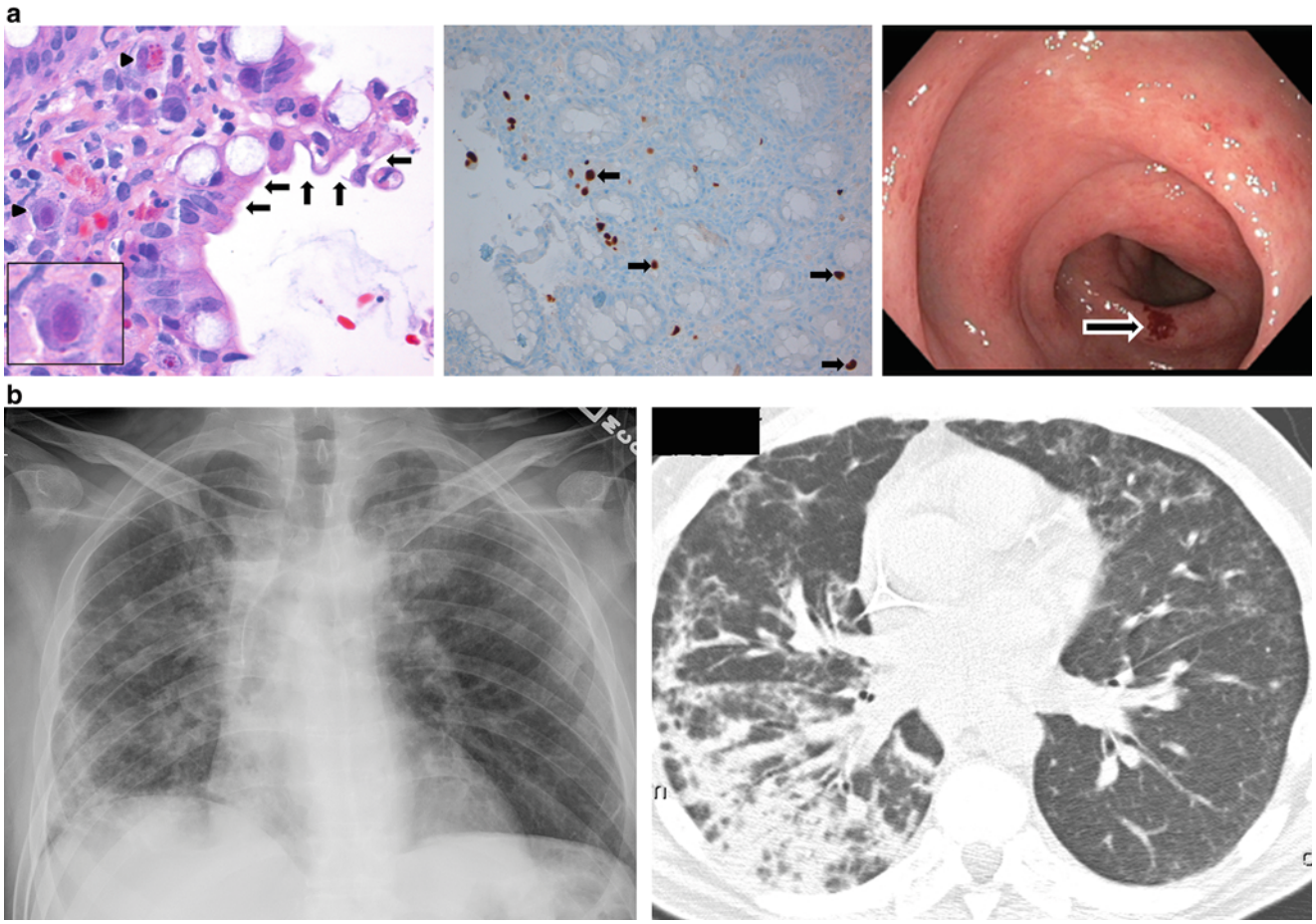

FIGURE 24-1. (**a**) CMV colitis in a CBT recipient. *Left panel*: histopathologic examination of ulcer biopsy specimen showing loss of superficial mucosal integrity (*arrows*) and viral inclusions (*arrowheads*). Inset shows higher magnification view of viral inclusion. *Middle panel*: immunohistochemistry demonstrating CMV-infected cells in biopsy specimen using an antibody recognizing the CMV gB protein. *Right panel*: endoscopic visualization of mucosal ulceration (*arrow*). Microscopy images courtesy of Dr. John Fortune, Department of Pathology, Oregon Health and Science University. (**b**) Chest X-ray (*left panel*) and computed tomography (*right panel*) of an allogeneic HCT recipient demonstrating bilateral interstitial infiltrates typical of CMV pneumonia.

107]. qPCR values of circulating CMV in plasma versus whole blood in a given patient may vary [114]; therefore, it is important to use the same blood component for testing when following serial viral loads. Although PCR has been used on BAL fluid [115], viral-load cutoffs have not been defined, and while the sensitivity and negative predictive values are very high, the specificity and positive predictive values are not known. Similarly, the significance of detection of CMV DNA by PCR in tissue samples such as lung, colon, or liver biopsy specimens for the diagnosis of CMV end-organ disease is not well established and will require further development and evaluation. PCR testing of CSF is specific and strongly indicative of CMV replication in the CNS. PCR testing of vitreous fluid strengthens the diagnosis of CMV retinitis.

The detection of CMV mRNA by PCR amplification on blood samples is equivalent to utilizing DNA PCR or p65 antigenemia to guide preemptive therapy after HCT [116, 117]. However, this method has not been as widely adopted as DNA-based PCR assays.

24.4 Clinical Manifestations

Defining the fundamental concepts of CMV "infection" and "disease" has been tremendously useful in the care of the individual patient and also in patient-centered clinical research. First developed and published in 1993, CMV definitions were updated in 1995 and 2002 to reflect advances in diagnostics and recognition of the "indirect effects" of CMV infection [118]. CMV "infection" simply indicates the detection of CMV, typically by DNA or messenger ribonucleic acid (mRNA) PCR, or pp65 antigenemia, from plasma or whole blood. CMV "disease" was

historically limited to "proven," as defined by the presence of symptoms and signs compatible with CMV end-organ involvement along with the detection of CMV in tissue from the relevant organ by histopathology, immunohistochemistry, or DNA hybridization [118]. Only retinitis was defined based solely on symptoms and/or signs when assessed by an experienced ophthalmologist. These definitions are being revised and expanded to include "probable" disease categorization based on new diagnostic techniques, primarily PCR-based (Table 24-1). Since CMV infection and disease are generally managed differently (see below), distinguishing between the two is critical.

24.4.1 Direct Effects

Almost any organ can be affected by CMV in the HCT recipient. Since the introduction of effective antivirals such as ganciclovir and sensitive monitoring techniques such as PCR, the overall incidence of CMV disease in the first year after HCT has fallen from approximately 30–35 to 5–10% among seropositive recipients [119]. Gastrointestinal disease and pneumonia are the most common manifestations of CMV end-organ disease after HCT.

Pneumonia is the most important clinical manifestation of CMV disease due to its high associated mortality. Prior to the

Table 24-1. Definitions of CMV disease in HCT recipients

	Classification	
Disease manifestation	Proven[a]	Probable[a]
Pneumonia	Tissue CMV positive by: Immunohistochemistry or Histopathology or DNA hybridization *Or* BAL: culture/shell vial	BAL or lung tissue CMV positive by: qPCR value above established threshold
Gastrointestinal[b]	Macroscopic mucosal lesions *And* Tissue CMV positive by: Immunohistochemistry *or* Histopathology *or* DNA hybridization	Tissue CMV positive by: Immunohistochemistry *or* Histopathology *or* DNA hybridization
Hepatitis	Abnormal serum transaminases *And* Tissue CMV positive by: Immunohistochemistry *or* Histopathology *or* DNA hybridization *And* Absence of other cause of hepatitis	Not defined
Retinitis	Ophthalmological signs[c] Vitreal fluid CMV PCR positive[d]	Not defined
CNS[e]	Tissue CMV positive by: Immunohistochemistry *or* Histopathology *or* DNA hybridization *or* Culture *or* PCR	CSF CMV positive by PCR[f]

[a]Both require the presence of the appropriate symptoms and/or signs of CMV disease.
[b]Esophagitis, gastritis, small or large bowel disease.
[c]As determined by an experienced ophthalmologist.
[d]Use as supporting evidence if clinical presentation is atypical.
[e]Ventriculitis, encephalitis.
[f]Requires absence of significant (visible) bloody contamination in CSF sample obtained.

development of effective preemptive and prophylactic strategies, the incidence of CMV pneumonia ranged from 1 to 6% after autologous HCT and 10 to 30% after allogeneic HCT [120]. Currently, CMV pneumonia accounts for approximately one-third of the cases of CMV disease [121]. The vast majority of cases occur after allogeneic HCT and typically within the first 60 days, but up to 30% of cases occur after day +100 [109, 122]. CMV pneumonia often manifests with fever, nonproductive cough, and hypoxia. It is important to recognize that fever may be absent in patients receiving high-dose immune suppression. The onset of symptoms can occur over 1–2 weeks, often times with rapid progression to respiratory failure and the requirement for mechanical ventilation. Although there are no specific radiologic changes, the most common radiographic findings consist of bilateral, ground-glass interstitial infiltrates (Figure 24-1b); small centrilobular nodules and air-space consolidations may also be present [123, 124].

The diagnosis of CMV pneumonia is established ("proven") by detection of CMV by shell-vial, culture, or histology in BAL or lung biopsy specimens in the presence of compatible clinical signs and symptoms (Table 24-1). Pulmonary shedding of CMV is common, but CMV detection in BAL by shell vial assay from asymptomatic patients who underwent routine BAL screening at day 35 after HCT was predictive of subsequent CMV pneumonia in approximately two-thirds of cases [125]. In centers where these techniques are no longer available, quantification of CMV DNA by qPCR in BAL fluid at a level above the threshold established by the center is indicative of "probable" CMV pneumonia (Table 24-1). Due to the high negative predictive value afforded by its high sensitivity, a negative PCR result can be used to rule out the diagnosis of CMV pneumonia [115].

Prior to effective antiviral therapy, the mortality rate of CMV pneumonia after HCT approached 100% [126]. The introduction of agents with potent anti-CMV activity resulted in improved outcomes but mortality rates remain in the range of 30–50% [126–131]. In the current era of preemptive antiviral therapy, lymphopenia and requirement for mechanical ventilation predict both overall and infection-attributable mortality [131].

Gastrointestinal disease is now the most common end-organ manifestation of CMV infection after HCT [104]. As with pneumonia, most cases occur within the first 3 months after allogeneic HCT [132]; however, direct infection-attributable mortality with GI tract disease is uncommon.

Any part of the gastrointestinal tract can be affected, from the esophagus to the colon. Esophagitis typically results in odynophagia, while gastritis often presents with epigastric abdominal pain and nausea. Hematochezia, diarrhea, and diffuse abdominal pain may occur with colitis. As none of these symptoms are pathognomic for CMV infection, endoscopy with tissue biopsy of abnormal areas is required for diagnosis (Table 24-1). Ulcers are often seen on endoscopy (Figure 24-1a, right panel), and visual differentiation of these lesions from other processes that may affect the gastrointestinal tract in these populations, such as graft-versus-host disease (GVHD), is often difficult. Therefore, the diagnosis of gastrointestinal disease ultimately relies on detection of CMV in biopsy specimens by histology combined with immunohistochemistry or DNA hybridization techniques (Figure 24-1a, left and middle panels) or with viral culture (if available). Notably, gastrointestinal disease can occur in the absence of CMV detection in the blood [105, 106, 133]. It should also be noted that GVHD and CMV gastrointestinal disease frequently occur together and therefore each condition's relative contribution to the patient's symptoms might be difficult to assess.

CMV hepatitis is less common than GI tract disease. Based on presenting features alone, it is difficult to distinguish hepatitis caused by CMV from other causes of hepatitis encountered after HCT, including GVHD. Therefore, liver biopsy is required to establish the diagnosis.

Retinitis is relatively uncommon after HCT [134–137]. Patients will often present with decreased visual acuity or blurred vision, and approximately 60% will have involvement of both eyes [135]. Most cases present later than day 100 after transplantation and are associated with prior CMV reactivation, delayed lymphocyte engraftment, and GVHD [135]. The diagnosis of CMV retinitis can often be made by an experienced ophthalmologist based on signs and symptoms alone. Detection of CMV in vitreal fluid by PCR can give supportive evidence for the diagnosis (Table 24-1).

CMV infection of the central nervous system (CNS) is less common after HCT than in the setting of advanced human immunodeficiency virus (HIV) infection. As opposed to pneumonia and GI tract disease, the onset of CNS disease is often late (after day +100) after HCT [138]. The most common disease manifestations are typical of encephalitis, with cognitive dysfunction and confusion [138–140]. The diagnosis of CMV CNS disease is made by detecting CMV in cerebrospinal fluid (CSF) by PCR or culture, or in brain tissue by culture or histopathology, in the appropriate clinical setting (Table 24-1) [118].

CMV rarely causes end-organ disease including, but not limited to, nephritis, cystitis, pancreatitis, and myocarditis; these additional disease categories are defined by the presence of compatible symptoms and signs, and documentation of CMV by biopsy.

24.4.2 Indirect Effects

In addition to the direct end-organ effects of CMV infection, CMV appears to be associated with consequences indirectly related to active infection [141]. After HCT, CMV infection has been associated with an increased risk of invasive bacterial and fungal infections [142]. CMV infection has also been suggested to be a risk factor for subsequent both acute and chronic GVHD after HCT [143–145] similar to the associative finding with rejection after solid organ transplant [146–149]. These findings have been attributed largely to modulation of the host immune system during infection.

Recently, there has been great interest in the role of CMV on disease relapse after HCT. The first hint of an effect came with the observation that patients with CMV infection had less relapse of leukemia compared with patients who had no CMV infection after BMT [150]. This finding was confirmed in a pediatric population in which CMV donor (D) seronegative/recipient (R) seronegative HCT was associated with an increased risk of relapse compared to D+ or R+ HCT [151]. A subsequent study in adults undergoing allogeneic HCT for AML found that early CMV reactivation was associated with a significant reduction in risk of leukemic relapse at 10 years after HCT [152]. Evaluation of a larger cohort of adults found that CMV reactivation was associated with a decreased risk of relapse at day +100 among patients with AML, and was associated with a decreased risk of relapse at 1 year in all patients when analyzed together [153]. Finally, a protective effect of CMV reactivation on relapse was observed in a small cohort of patients who underwent transplant for CML [154]. A large CIBMTR study assessing CMV infection and relapse after HCT is now underway. The mechanisms underlying these findings are poorly understood. An interesting hypothesis is that CMV reactivation stimulates γδ T cells that cross-recognize leukemic cells [155]. Other proposed mechanisms are through stimulation of NK-cell mediated clearance of leukemic cells, or by direct induction of apoptosis in leukemic cells [156–159]. However, any potential benefit of CMV reactivation in terms of disease relapse is almost certainly outweighed by the negative effect of CMV serostatus and reactivation on non-relapse and overall mortality [104, 153, 160–162].

24.5 Risk Factors for CMV Infection and Disease

24.5.1 Allogeneic HCT Recipients

In the setting of allogeneic HCT, the most important risk factor is the serological status of the donor and recipient [161]; both should be routinely assessed prior to HCT. CMV D−/R− transplants have a very low risk of primary infection in the recipient. Primary infection can still occur if CMV is transmitted in transfused blood products or is acquired via contact with another individual with active CMV infection.

Approximately 20–30% of seronegative recipients who receive stem cells from a seropositive donor will develop primary CMV infection due most likely to transmission of latent CMV via the allograft [163, 164]. The risk of transmission is directly related to the allograft nucleated white blood cell count [163], consistent with hematopoietic myeloid lineage cells acting as a reservoir of latent CMV [2]. CMV D+/R− mismatching negatively impacts the overall survival and increases the transplant-related mortality, especially those caused by bacterial and fungal infections [142, 165]. More recent studies performed in the modern diagnostic and therapeutic era have confirmed the negative effect of CMV D+/R− mismatching [160]. Recently, a large study found a strong negative effect on overall survival, relapse-free survival, and transplant- related mortality in CMV D+/R− *unrelated* HCT but a much smaller negative effect for human leukocyte antigen (HLA)-identical sibling D+/R− HCT, and no effect in patients receiving mismatched related donor grafts [166]. Thus the impact of D+/R− sero-mismatching may be influenced by the type of HCT.

Without prophylaxis, approximately 60–70% of CMV-seropositive patients will experience CMV infection after allogeneic peripheral blood or bone marrow HCT. It is well-established that a CMV-seropositive recipient is at higher risk for mortality than a seronegative recipient after HCT [167–170].

Unlike the situation of D+/R− HCT, in which the negative impact of a seropositive donor is well-described, the impact of donor serostatus when the recipient in seropositive has been the subject of controversy. Some studies reported a beneficial effect of having seropositive donor with regard to a reduction in relapse- or nonrelapse-related mortality (NRM), whereas other studies found no such benefit [117, 151, 169, 171–179].

To reconcile these differences, a large retrospective analysis of over 29,000 patients from the European Society for Blood and Marrow Transplantation (EBMT) registry was performed [166]. Seropositive patients receiving grafts from seropositive unrelated donors had improved overall survival compared with seronegative donors if they had received myeloablative, but not reduced-intensity, conditioning, perhaps due to loss of CMV-specific T cell function after myeloablative conditioning. No effect was observed when they received allograft from HLA identical sibling donors. Thus, the negative effect of CMV D−/R+ mismatching may be limited to high-risk transplant settings.

In addition to the effects on non-relapse mortality and overall survival, the D−/R+ serological combination has been reported as a risk factor for delayed CMV specific immune reconstitution [180–183], CMV reactivation [181, 184], late CMV recurrence [185], and CMV disease [113, 181, 186].

Other risk factors for CMV infection after allogeneic HCT include the use of steroids at doses greater than 1 mg/kg body weight/day, T-cell depletion (either ex vivo or in vivo), acute and chronic GVHD, the use of total body irradiation $CD4^+$ lymphopenia, and the use of mismatched or unrelated donors [110, 113, 186–190]. Whether the source of stem cells (peripheral blood versus bone marrow) has a significant impact on the development of CMV infection and disease is not clear, as several studies have yielded conflicting results [186, 190–192]. Interestingly, the use of sirolimus for GVHD prophylaxis appears to protect against CMV infection, possibly due to the inhibition of cellular signaling pathways that are co-opted by CMV during infection for synthesis of viral proteins [186, 193, 194].

The use of HLA-matched, related nonmyeloablative conditioning regimens generally results in a less CMV infection and disease early after HCT compared to standard myeloablative regimens [195]. However, by 1 year after HCT, the risk of CMV infection and disease is equal among nonmyeloablative and myeloablative groups [194, 196].

Umbilical cord blood transplantation (CBT) is a technique that is now utilized when a suitable donor for bone marrow or peripheral blood stem cell transplantation is not available [197]. Since most infants are born without CMV infection, the transplanted allograft is almost always CMV-negative. CMV seropositive CBT recipients are at particularly high risk for infection compared to GSCF-mobilized peripheral blood stem cell transplant recipients due to delayed T cell immune reconstitution [198] and failure of functional CMV-specific T cells to achieve sufficient numbers to control CMV reactivation after CBT [29]. The reported rate of CMV reactivation after CBT varies widely, from 21 to 100%, while disease occurs in ~5–28% of recipients [190, 199–208]. The variability in reported infection rates likely reflects differences in conditioning regimens, inclusion of low-risk CMV seronegative recipients in certain data cohorts, and approaches to CMV prevention after CBT. One center reported a markedly high rate of CMV disease, particularly during the pre-engraftment period, and associated mortality after CBT [205], prompting a change in their preventative approach after CBT (discussed below).

An alternative stem cell source for patients who do not have matched donors is the HLA-haploidentical 2 or 3-loci mismatched family donor [209]. Such haploidentical transplantation has traditionally been associated with a high incidence of severe GVHD and graft rejection, prompting the implementation of T cell depletion strategies to reduce these adverse alloreactive events [209]. While T cell depletion does prevent GVHD, the consequent delayed immune reconstitution led to increased risk of infection [210–212]. High rates of CMV disease, antiviral drug resistance, and infection-attributable mortality have been reported in this population [213]. Performing T-cell-replete haploidentical HCT with posttransplant cyclophosphamide to induce immune tolerance [214] may reduce the incidence of CMV infection and disease compared to T-cell depletion [215, 216].

24.5.2 Autologous HCT

After autologous transplantation, approximately 40% of seropositive patients will have detectable CMV infection [217, 218]. While CMV disease is rare after autologous transplantation [191, 219–221], the outcome of CMV pneumonia is similar to that after allogeneic HCT [217, 222, 223]. Risk factors for CMV disease after autologous transplantation include CD34+ selection, high-dose corticosteroids, and the use of total-body irradiation or fludarabine as part of the conditioning regimen [191]. Therefore, while CMV is not typically considered a significant pathogen after autologous HCT, certain patients who are at high risk for CMV in this setting merit routine surveillance and preemptive therapy.

24.5.3 Late CMV Infection After Allogeneic HCT

Whereas CMV was typically seen by 100 days after allogeneic HCT [224], it has become recognized as a significant problem after day 100 as well [109, 185, 225]. Several factors predict the development of late CMV infection, including prolonged or repeated CMV infection and/or disease before day +100, use of antiviral prophylaxis during the early posttransplant period, slow response to antiviral therapy, qualitative or quantitative lymphopenia, cord blood transplants, patients with severe acute or chronic GVHD, and HLA-mismatched transplant [19, 20, 109, 113, 185, 186, 226]. Patients, who have experienced prolonged or repeated CMV episodes before day 100, cord blood transplant recipients, and patients with significant immunosuppression should have continued weekly surveillance to reduce the risk of late CMV disease.

24.6 Antiviral Agents

Agents licensed for the treatment or prevention of CMV infection include ganciclovir (GCV) and its oral prodrug valganciclovir (vGCV), foscarnet (FOS), and cidofovir (CDV) (Table 24-2). All exert their antiviral effect by inhibiting viral DNA synthesis through targeting of the viral DNA polymerase encoded by the UL54 gene. Acyclovir (ACV) and its oral prodrug valacyclovir (vACV) do not possess potent activity against CMV and therefore cannot be used for treatment of infection but have shown efficacy when used as prophylaxis (discussed below).

Table 24-2. Agents licensed for the treatment or prevention of CMV infection and disease

Agent	Target	Route of administration	Dose[a]		Toxicities[b]	Resistance mutations
			Induction	Maintenance		
Ganciclovir	UL54	IV	5 mg/kg bid	5 mg/kg/day	Neutropenia, anemia, thrombocytopenia, diarrhea, fever	UL97, UL54
Valganciclovir	UL54	oral	900 mg bid (≥40 kg)	900 mg/day (≥40 kg)	Same as ganciclovir	UL97, UL54
Foscarnet	UL54	IV	90 mg/kg bid	90 mg/kg/day	Nephrotoxicity, electrolyte, wasting, nausea, urethral ulceration, paresthesia, hallucination	UL54
Cidofovir	UL54	IV	5 mg/kg/week	5 mg/kg every other week	Nephrotoxicity, neutropenia, headache, nausea, uveitis/iritis, diarrhea, ocular hypotony	UL54

[a]All agents require dose adjustment in the setting of renal dysfunction.
[b]For full listing of toxicities, please refer to the summary of product characteristics (SPC) for each agent.

GCV is a nucleoside analogue of guanosine that acts as a competitive inhibitor of deoxyguanosine triphosphate incorporation into viral DNA by the viral DNA polymerase UL54. A CMV gene, UL97, encodes a kinase that phosphorylates GCV to GCV monophosphate, a necessary step in conversion of GCV to its active form. Cellular kinases then phosphorylate GCV monophosphate to the active triphosphate form. GCV is currently the first-line agent for CMV prophylaxis, preemptive treatment, and treatment of CMV disease, barring contraindications. Neutropenia occurs in up to 30% of HCT recipients during GCV therapy [227], thereby placing the patient at risk of invasive bacterial and fungal infections [227, 228]. vGCV achieves serum concentrations at least equivalent to intravenous GCV [229, 230] and the toxicity profile appears similar. However, drug levels can be unpredictable, especially in patients with gastrointestinal tract GVHD, and therapeutic drug monitoring can therefore be a useful tool in managing patients on vGCV therapy. Neutropenia often responds to dose reduction and support with granulocyte-colony stimulating factor, but occasionally discontinuation of GCV or vGCV is required, in which case FOS is typically the second-line agent of choice.

FOS is a pyrophosphate analogue that binds directly to and competitively inhibits the CMV DNA polymerase UL54. Although a randomized, controlled trial showed similar efficacy and rate of side effects for GCV and FOS when used as preemptive therapy [231], practical issues such as the need for intensive hydration along with the electrolyte wasting that accompany FOS have resulted in its use mostly as a second-line agent when GCV or vGCV are contraindicated or not tolerated, or there is suspicion of GCV resistance (see below).

CDV is a cytosine nucleotide analogue that, like FOS, does not require phosphorylation by the CMV UL97 kinase for antiviral activity. Instead, cellular enzymes convert CDV to CDV triphosphate, which then inhibits the CMV DNA polymerase. The long half-life of cidofovir allows a once-per-week dosing schedule. However, the major toxicity with CDV—renal tubular damage—limits its utility after HCT and it should therefore be considered third-line therapy after GCV and FOS.

24.6.1 Antiviral Resistance

Drug resistance is relatively uncommon after peripheral blood or bone marrow HCT [232] but the risk has been reported to be increased after T-cell-depleted haploidentical HCT [213]. Resistance typically occurs in the setting of ongoing, intermittent or recurrent viral replication in the presence of drug. This situation arises most often due to profound host immunosuppression and/or suboptimal drug levels. Therefore, reducing immune suppression and optimization of drug delivery are important aspects of management. CBT or T-cell-depleted transplant recipients and those on augmented immune suppression for GVHD should be considered at increased risk for resistance. Inadequate drug delivery may occur in a patient receiving vGCV during GI GVHD, or when dosages are improperly adjusted for renal dysfunction. When available, therapeutic drug level monitoring may be of benefit.

Drug resistance should be suspected in patients with some or all of the above risk factors who have a rising viral load after at least 2 weeks of antiviral therapy or who experience worsening or relapse of clinical disease or viremia while on prolonged therapy. In general, resistance requires accumulated drug exposure; in treatment-naïve patients, no decrease or even a moderate increase in the viral load will occur in many patients within the first 2 weeks of starting therapy that is likely due to the underlying immunosuppression, not true

drug resistance [103]. Thus, this situation does usually not warrant change of therapy. The duration of drug exposure required to select for resistance and the increase in viral load that should prompt testing for resistance after HCT are not well defined and likely depend on the above mentioned host factors, viral loads during therapy, and genetic barrier to resistance for the drug in question.

Since GCV/vGCV is typically used as a first-line agent for CMV infection and disease, resistance to this antiviral is the most commonly encountered problem. A general approach to the patient with suspected GCV resistance is presented in Figure 24-2. GCV resistance is usually due to mutations in the UL97 gene; mutations in UL54 may follow UL97 mutations with continued GCV exposure. UL97 and UL54 mutations that confer GCV resistance have been determined and genotypic assays are available for diagnostic analysis in reference laboratories [232]. Since different UL97 mutations confer varying degrees of GCV resistance, some cases of genotypically defined GCV -resistant CMV may still respond to high-dose GCV therapy (i.e., twice standard induction dose) if they confer low-level (two- to threefold) resistance [232]. However, if there is evidence of CMV disease or the viral load is increasing rapidly, a switch to FOS is recommended [232].

Since neither FOS nor CDV activity are dependent on phosphorylation by the UL97 gene product, CMV that has acquired GCV resistance due to UL97 mutations will still be susceptible to these agents. Due to its relatively favorable toxicity profile compared to CDV, FOS is most often used as the agent of choice in the setting of GCV resistance. Studies evaluating the utility of combination therapy of FOS and GCV for GCV-resistant CMV disease have been inconclusive, and therefore, this strategy is not routinely recommended [233].

Mutations in UL54 may confer resistance to GCV, FOS, CDV, or cross-resistance to combinations thereof. Cross-resistance between FOS and GCV due to UL54 mutations rarely occurs, while on the other hand most UL54 mutations that confer GCV resistance also result in CDV resistance [232]. Rarely, mutations in UL54 that confer resistance to all three agents—GCV, FOS, and CDV—are encountered [232]. Therapeutic options in such situations are limited and highlight the need for antiviral agents with targets other than UL54. The use of a sirolimus-based regimen for GVHD prophylaxis may provide some benefit for reasons discussed above but should be viewed as an adjunct to, not a substitute for, direct antiviral therapy.

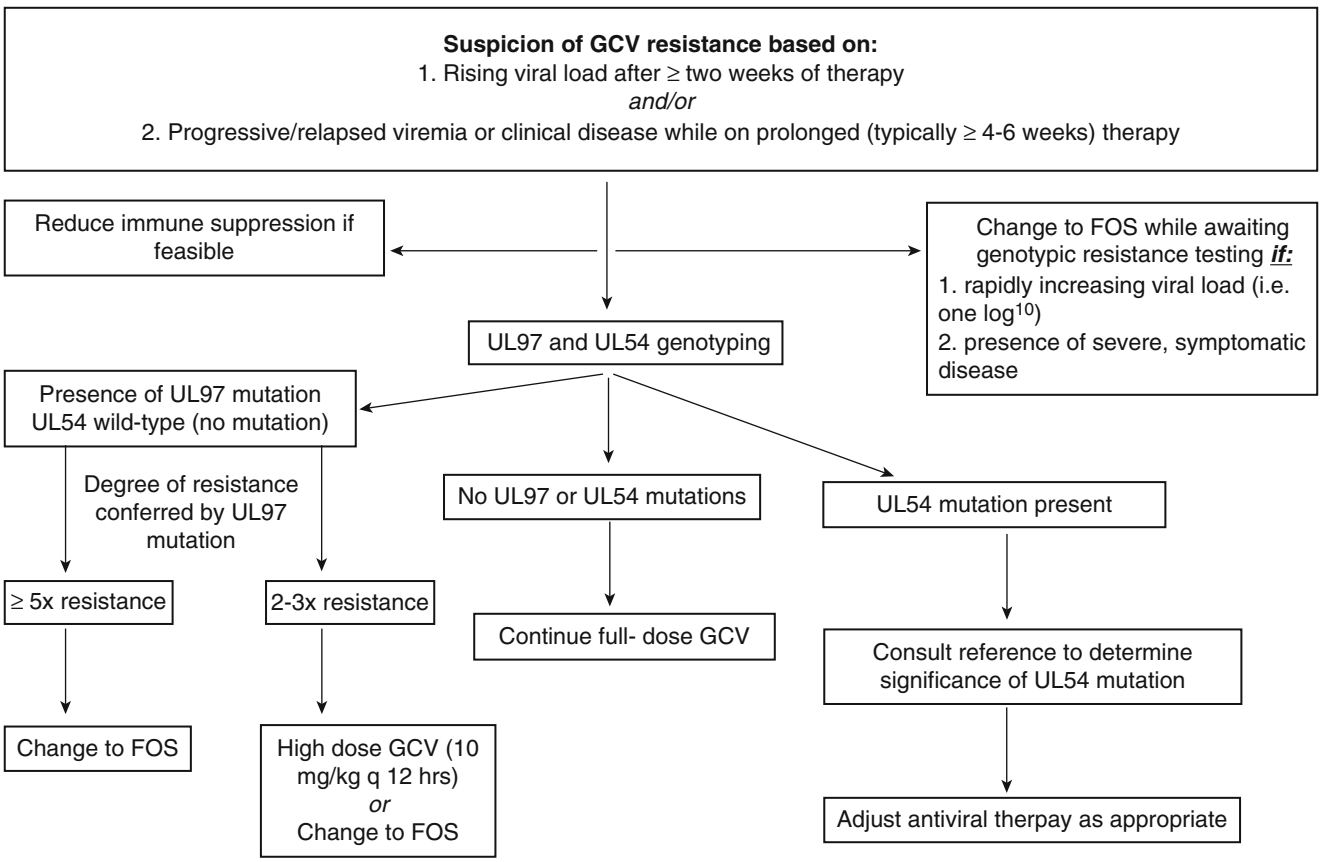

FIGURE 24-2. Approach to the patient with suspected GCV resistance.

24.6.2 Antiviral Agents in Development

Maribavir (MBV) (Table 24-3) is an oral agent that inhibits the CMV UL97 kinase and potently inhibits CMV replication in vitro [234]. Due to its mechanism of action, MBV is active against CMV strains resistant to GCV, FOS, and CDV [235] but antagonizes the antiviral activity of GCV [236]. After promising results phase I and II clinical trials, MBV failed to effectively prevent CMV infection compared to placebo after HCT when used as prophylaxis in a phase III trial [237–239]. The reason(s) underlying the failure of MBV in the phase III study are not clear but the use of too low a dose of MBV is often cited [240]. A phase II dose-ranging trial comparing higher doses of MBV to standard of care GCV (or vGCV) as preemptive therapy after allogeneic HCT (EudraCT: 2010-024247-32) has been completed. In addition, MBV has demonstrated efficacy in the treatment of refractory or resistant CMV infections after transplantation [241, 242] and a phase II study for this indication has been completed (ClinicalTrials.gov NCT01611974). Results from these two phase II trials are forthcoming. MBV resistance due to mutations in UL97 has occurred in patients treated with this agent [243, 244].

Letermovir (Table 24-3) inhibits the activity of the essential CMV UL56/UL89 DNA terminase complex [245]. Letermovir is active against wild-type and drug-resistant CMV in tissue culture [245]. Experience using letermovir for multidrug-resistant CMV disease in vivo is promising but very limited [246]. A phase II study of letermovir as prophylaxis in CMV-seropositive HCT recipients showed a dose-dependent reduction of prophylaxis failure (defined as discontinuation of letermovir or placebo because of CMV antigen or DNA detection, end-organ disease, or any other cause) compared to placebo [247]. A phase III randomized multicenter trial as prophylaxis in seropositive HCT recipients has completed patient enrollment (ClinicalTrials.gov NCT02137772). Letermovir appears to be very well tolerated, with few demonstrable side effects or toxicities [247]. Resistance mutations mapping to UL56 can be selected for in tissue culture [248]; whether similar mutations will be arise in patients treated with letermovir remains to be seen. Demonstration of an additive antiviral effect when combined with DNA polymerase inhibitors [249] raises the possibility of combination therapy similar to strategies currently employed for the treatment of hepatitis C and HIV.

Brincidofovir (CMX-001) (Table 24-3) is a lipid-conjugated nucleotide analogue of CDV that has a high oral bioavailability and long half-life. It has activity against most DNA viruses, including CMV [250]. In contrast to CDV, brincidofovir is not associated with significant nephrotoxicity. Brincidofovir at a dose of 100 mg twice daily was shown to be effective in preventing CMV infection after HCT when used as prophylaxis in a phase II placebo-controlled study [251]. However, diarrhea and acute gastrointestinal GVHD were reported more frequently in the group that received this dose compared to placebo or lower dose brincidofovir, and gastrointestinal side effects were dose-limiting at 200 mg twice weekly. A phase III randomized multicenter trial using a dose of 100 mg twice weekly as prophylaxis in seropositive HCT recipients (ClinicalTrials.gov NCT01769170) has been completed, and results are forthcoming. While resistance to brincidofovir has not been well characterized, it is expected that mutations in UL54 conferring CDV resistance will also result in brincidofovir resistance [252].

While reports of leflunomide and the antimalarial artesunate having anti-CMV activity exist [253–255], neither of these have conclusively demonstrated benefit and are not approved by European or American regulatory authorities for the treatment of CMV; therefore, their routine use cannot be recommended. The immunosuppressive drug sirolimus inhibits CMV replication in tissue culture by regulating key cellular signaling pathways and has been shown to reduce the risk of CMV reactivation after HCT and renal transplantation [186, 193, 256]. Thus, this agent may be a useful adjunct when ongoing immune suppressive therapy is required in the setting of refractory CMV infection.

TABLE 24-3. CMV antiviral agents in development

Agent	Target	Route of administration	Dose	Toxicities	Resistance mutations
Maribavir	UL97 kinase	Oral	400–1200 mg twice daily	Taste disturbance	UL97, UL27[a]
Letermovir (AIC-246)	UL56/UL89 terminase complex	Oral, IV	240 mg daily[b], 480 mg daily[c]	None apparent	UL56[a]
Brincidofovir (CMX-001)	UL54 DNA polymerase	Oral	100 mg twice weekly[b]	Gastrointestinal[d]	Not described

[a]Found only in tissue culture thus far.
[b]Dose chosen for phase III studies.
[c]In patients receiving cyclosporine for GVHD prophylaxis.
[d]Diarrhea, nausea, vomiting, abdominal pain, aGVHD, elevated ALT.

24.7 Prevention of Infection and Disease

24.7.1 Choice of Donor

Recipients who are CMV seronegative before allogeneic HCT should ideally receive a graft from a CMV seronegative donor to prevent primary infection via the allograft. No data exists indicating whether HLA-matching is more important compared to CMV serostatus in affecting a good outcome for the patient. Given the choice, an antigen-matched donor for HLA-A, B, or DR would most likely be preferred to a CMV-negative donor. For lesser degrees of mismatch, (allele-mismatches or mismatches on HLA-C, DQ, or DP), the CMV-serostatus of donor should be considered a factor even if the match was poorer. Compared to other donor factors such as donor age or blood group, a CMV-seronegative donor would have preference. If the patient is CMV seropositive, it has been shown that a CMV seropositive unrelated donor confers a survival advantage if the patient will receive myeloablative conditioning [166]. Similar to the situation with a CMV seronegative patient, an antigen-match on A, B, and DR is the major selection criterion but CMV-status should be weighed among other factors with lesser degrees of HLA-mismatch.

24.7.2 Transmission via Blood Products

Previously, the transfusion of blood products represents a significant source for CMV infection in seronegative transplant recipients. Today preventive measures such as using blood products from CMV seronegative donors or leukocyte-reduced, filtered blood products are widely used and greatly reduce this risk [257–259]. It is not clear which strategy is the most effective [260, 261] and no controlled study has investigated whether there is an extra benefit from the use of both methods.

24.7.3 Immune Therapy

Intravenous immune globulin (IVIG) is not reliably effective as prophylaxis against primary CMV infection. One study demonstrated a reduction in the rate of CMV infection but not disease, while another study was unable to confirm protection from infection [262, 263]. Similarly negative results were observed using a CMV-specific monoclonal antibody [264]. Likewise, the effect of immunoglobulin on reducing CMV infection in seropositive patients is modest, and no survival benefit among those receiving immunoglobulin has been reported in any study or meta-analysis [265–270]. Therefore, the prophylactic use of IVIG is not recommended.

24.7.4 Chemoprevention

The strategies of prophylactic or preemptive use of antiviral agents after HCT have markedly reduced the incidence of CMV disease and improved survival among at-risk populations. All centers performing allogeneic transplants should therefore have one of these strategies in place for all allogeneic HCT recipients at risk for CMV infection (seropositive recipients, or seronegative recipients of a seropositive donor graft) [271]. Studies in the eras of pp65 and qPCR monitoring have documented the equivalence of prophylaxis and preemptive therapy in terms of preventing CMV infection and disease after HCT [127, 272]. Most transplant centers have moved towards preemptive strategies as pp65 antigenemia and qPCR-based diagnostics techniques have become readily available [273]. DNA qPCR has become the standard for monitoring at many institutions as it is more sensitive than pp65 antigenemia [127] and technically easier to perform than mRNA detection. Additionally, it has been reported that qPCR-based initial viral load and viral load kinetics are important as risk factors for CMV disease [111].

Prophylaxis denotes the routine administration of antivirals to all at-risk patients regardless of the presence of active CMV infection, typically until day +100 after HCT. ACV and its vACV, while not approved for the treatment of CMV, are used at some centers for CMV prophylaxis after HCT [273]. High dose ACV and vACV have demonstrated efficacy in reducing the risk for CMV infection and disease after HCT [220, 274–276]. Routine monitoring for CMV infection is still required if vACV or ACV prophylaxis is used, and therapy with GCV or vGCV is indicated if CMV is detected. GCV prophylaxis, begun at engraftment and continued until day +100, has been demonstrated to reduce the risk of CMV infection and disease after HCT compared to placebo, although its use is limited by toxicity, primarily marrow suppression [127, 228, 277]. Data regarding vGCV prophylaxis is more limited. A recent randomized, double-blind study of vGCV prophylaxis compared to preemptive therapy for the prevention of late CMV infection after HCT demonstrated reduced CMV viremia in the prophylaxis group but no difference in CMV disease [272].

Preemptive therapy, on the other hand, withholds antiviral therapy until CMV infection is detected in whole blood or plasma samples. This strategy mandates sensitive, specific, and rapid turnaround laboratory tests to detect circulating CMV in order to enable initiation of antiviral therapy prior to the development of CMV end-organ disease. All patients who have undergone allogeneic HCT should be monitored at least once per week beginning either at the time of transplant or ~day +10 and extending to at least day +100 after HCT [271]. Surveillance should be extended past day +100 in those at risk for late infection and disease

(discussed above). The ideal duration and frequency of CMV monitoring in the later transplantation periods have not been defined [195, 278].

Although CMV infection is rare in D−/R− patients, such a monitoring strategy is effective in identifying CMV infection and preventing disease in a large cohort of such patients [279]. Routine monitoring of autologous HCT recipients is not recommended, with the exception being high-risk patients as described above.

In all patients in whom viremia is detected, a thorough evaluation of the patient in order to assess for signs and symptoms of CMV disease is necessary. Initiation of induction-dose preemptive antiviral therapy is generally recommended [271]. However, it has been clearly shown that most patients with low viral loads can be safely spared preemptive antiviral therapy unless there are special high risk features [104, 113, 280]. Currently, there are no validated universal viral load thresholds for starting preemptive therapy, and such thresholds are difficult to establish due to differences in assay performance and testing material (i.e., whole blood versus plasma) [281]; the development of an international standard for CMV qPCR calibration [282] may eventually allow for this. Additionally, thresholds for initiating preemptive therapy need to account for underlying patient characteristics which determine the risk for progression to CMV disease.

Currently, considerable variation in practice exists pertaining to the duration of induction dose preemptive treatment [273]. In general, this should be continued for a minimum of 1–2 weeks and a decrease in viral load has been documented by qPCR, followed by maintenance therapy until the CMV viral load is undetectable [271] or below a center's established cutoff. After discontinuation of preemptive therapy, routine weekly screening until day +100 or later if risk factors for late infection are present are still necessary to monitor for recurrence of viremia [271]. If less sensitive markers than qPCR, such as the pp65 antigenemia assay, are used, then preemptive therapy should be continued until 2 negative assays are obtained [231].

GCV is considered the first-line agent for preemptive therapy [271]. While FOS has demonstrated equivalence to GCV when used in a preemptive manner [231], practical aspects of its administration relegate its use to situations when GCV is contraindicated or not tolerated. The results of several uncontrolled studies suggest that vGCV is comparable to intravenous GCV in terms of efficacy and safety when used as preemptive therapy after allogeneic HCT [283–288]. A prospective, randomized trial comparing vGCV to intravenous GCV supported these observations [289]. Thus, in the HCT recipient who is able to tolerate oral therapy and in whom no barriers to efficient absorption of an oral agent exist, vGCV appears to be a reasonable alternative to intravenous GCV for preemptive therapy.

There has been great interest in utilizing methods to determine CMV-specific immune reconstitution after HCT as an additional means to stratify risk of CMV infection and disease ("immune monitoring") and further tailor surveillance and preemptive therapy strategies. The types of assays used, their strengths and limitations, and their predictive value in terms of CMV infection and disease after transplantation have been extensively reviewed elsewhere [69, 290]. While promising, the use of immune monitoring in this fashion requires validation in large, randomized trials before it can be recommended.

24.7.5 Vaccination

Given the costs and toxicities associated with antiviral therapy, a vaccine to prevent CMV infection would be of substantial benefit. Indeed, the Institute of Medicine has given the development of a CMV vaccine highest priority [291]. Historically, most vaccine candidates yielded mixed results [292]. Recently, the safety and efficacy of a DNA vaccine expressing the CMV immunogenic proteins gB and pp65 was evaluated in a phase II, placebo controlled trial in CMV seropositive allogeneic HCT recipients [293]. While no difference in initiation of preemptive anti-CMV therapy or duration of antiviral therapy was observed between the groups, the group receiving the vaccine had fewer episodes of viremia, lower viral loads, and was more likely to be viremia-free at 1 year after HCT. No differences in CMV disease were observed but the overall incidence of disease was low (7.5% in vaccine group vs. 8.8% in placebo group). A phase III study of this vaccine in a similar patient population is currently underway (ClinicalTrials.gov NCT01877655). CMV peptide vaccines designed to elicit pp65-specific CTL were found to be safe and immunogenic in healthy adults [294] and a phase II study in HCT recipients is under way (ClinicalTrials.gov NCT02396134).

24.7.6 Special Populations

Patients with CMV disease occurring prior to planned allogeneic HCT have a very high risk of death after transplantation [295]. After transplantation, a patient with documented pretransplant CMV disease should either be monitored for CMV very closely (i.e., twice weekly), or be given prophylaxis with GCV or FOS.

The CMV seropositive CBT recipient population may benefit from more intensive prevention strategies. The reactivation rate in CMV seropositive CBT recipients in the absence of high-dose ACV/vACV or anti-CMV prophylaxis has been reported at 70–100% [205, 207, 208, 296]. A combination approach of high-dose vACV prophylaxis coupled with continued monitoring and preemptive therapy was

associated with rates of CMV reactivation and disease similar to those seen after allogeneic BMT or PBSCT [190]. Other studies have described successful vGCV or GCV prophylaxis and preemptive treatment strategies after CBT using protocols similar to other allogeneic HCT recipients [208, 297]. More recently, an aggressive approach of pretransplant GCV along with posttransplant high dose ACV/vACV prophylaxis and biweekly monitoring was demonstrated to reduce the incidence of CMV infection and disease after CBT [205]; the relatively contributions of these interventions towards CMV prevention are unclear. Thus, the optimal approach to CMV after CBT has not been determined.

24.8 Management of CMV Disease

As mentioned earlier, the diagnosis of CMV disease requires documenting the presence of CMV in the appropriate diagnostic specimen, coupled with symptoms and signs consistent with CMV. GCV is considered first-line therapy for end-organ disease, with FOS reserved as an alternative if neutropenia or other factors precluding GCV use are present. As opposed to preemptive therapy, the treatment of end-organ disease requires longer courses of induction-dosing antiviral therapy. For gastrointestinal disease, standard therapy generally entails induction treatment with an intravenous antiviral, most often GCV, for 3–4 weeks followed by several weeks of maintenance. Shorter courses of induction therapy (2 weeks) are not as effective [298]. Recurrence of GI disease may occur in approximately 30% of patients in the setting of continued immunosuppression and such patients may benefit from secondary prophylaxis with maintenance antivirals until immunosuppression has been reduced. Similar to GI tract disease, the treatment of CMV pneumonia involves induction-dose GCV for 3–4 weeks, followed by a period of maintenance therapy.

The role of vGCV in the management of CMV disease after HCT is not well established. vGCV has been shown to be noninferior to IV GCV in the treatment of non-life threatening CMV disease after solid-organ transplant, primarily kidney transplant recipients [299]. However, similar studies have not been performed in HCT recipients. Therefore, IV anti-CMV therapy remains the standard of care, although oral vGCV may be considered for patients with mild or moderate, non-life threatening disease after an initial period of IV therapy to bring disease under control and suppress viremia. In general, vGCV should only be used if there are no factors that would impair the absorption of an orally administered medication, such as severe gastrointestinal GVHD.

The role of IVIG as an adjunct to antiviral therapy for CMV disease remains controversial due to the lack of prospective, randomized trials evaluating the additional benefit of this intervention over antiviral therapy alone [122]. There does not appear to be a specific advantage of CMV-specific immune globulin (CMV-Ig) compared to pooled immunoglobulin [300]. While there is no role for IVIG in the treatment of gastrointestinal disease [301], it has been considered as standard-of-care at many centers in the management of CMV pneumonia based on small studies showing improved survival rates with the addition of IVIG compared to historical controls using antiviral therapy alone [302–304]. On the other hand, a recent, large retrospective analysis was unable to demonstrate an improvement in overall or infection-attributable mortality with the addition of IVIG to antiviral therapy [131]. Thus, the role of IVIG in the management of CMV pneumonia remains unclear.

CMV retinitis is typically treated with systemic therapy, with or without intraocular GCV injections or implants [135, 305–307]. The optimal duration of therapy is not well established, but in general longer courses are needed in order to prevent recurrence.

Other manifestations of CMV disease, such as hepatitis and encephalitis, are uncommon and are typically managed with intravenous therapy. The duration of therapy for these manifestations has not been well established and should be tailored to the individual patient.

24.9 Adoptive Immunotherapy

Due to the importance of CMV-specific functional T cells in the control of CMV infection after HCT [23], there has been intense interest in promoting CMV immune reconstitution via the adoptive transfer of CMV-reactive T cells [308]. This topic is discussed in more detail elsewhere in this book.

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25 Cytomegalovirus Infection After Solid Organ Transplantation

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25.1 Epidemiology and Pathogenesis

Cytomegalovirus (CMV) is a β-herpes virus that commonly infects humans [1–5]. The seroprevalence rates of CMV vary from as low as 50% in the USA and the developed world to as high as >95% in developing countries [1–5]. The seroprevalence rate increases with age, and reaches over 90% of adults >80 years of age [5]. Primary CMV infection is acquired from contact with infected body fluids such as saliva, urine, or genital secretions [6]. In immunocompetent individuals, primary CMV infection is mostly asymptomatic, although it may occasionally manifest clinically as an infectious mononucleosis-like syndrome characterized by fever and lymphadenopathy [6].

The immune response to CMV infection is initiated upon the recognition of CMV antigens by pathogen-recognition receptors, such as Toll-like receptors, expressed on macrophages, dendritic cells, and other innate immune cells [7–11]. This pathogen–immune cell interaction results in the secretion of interferon-γ, tumor-necrosis factor (TNF)-α, various interleukins, and other cytokines, and the upregulation of co-stimulatory molecules that orchestrates the development of adaptive CMV-specific cell-mediated and humoral immunity [9–13]. Despite a robust innate and adaptive immune response, CMV infection leads to lifelong latency and persistence in the host, with intermittent periods of subclinical reactivation that is controlled by CMV-specific memory cells [14–16].

Cells that allow for CMV latency and persistence are widely distributed, including hematopoietic progenitor cells [17], peripheral blood mononuclear cells [6, 18], polymorphonuclear leukocytes [19], and macrophages [20]. CMV also infects fibroblasts, smooth muscle cells, and endothelial cells [21]. The wide distribution of CMV-infected cells in tissues and organs, such as blood, liver, kidney, lungs, and heart allows for its efficient transmission during organ transplantation [22–25].

25.2 Mechanisms of Acquiring CMV Infection After Solid Organ Transplantation

The three major patterns for acquiring CMV infection after solid organ transplantation are (1) primary infection, (2) reactivation, and (3) superinfection.

25.2.1 Primary Infection (CMV D+/R− and D−/ R−)

Primary CMV infection occurs when a CMV-seronegative person (CMV R-) receives an organ from a CMV-seropositive donor (CMV D+). Since latent CMV is widely distributed, it is likely harbored in the allograft of CMV-seropositive donors. About 15–25% of solid organ transplantation in adults is in CMV D+/R− mismatched patients, and is characterized by efficient transmission of latent CMV from donor to the recipient [26–29]. Multiple viral strains may be transmitted from a single donor [30, 31]. Without any specific prevention strategy, this often leads clinically to CMV disease, which can be severe in nature [32].

Much less commonly, primary infection may occur in a CMV D−/R− transplant recipient who acquires CMV from transfusion of blood products from CMV-seropositive donors (transfusion-transmitted CMV infection) or through natural transmission routes in the community (i.e., contact with CMV-infected body fluids and secretions such as saliva) [33, 34]; the latter two mechanisms account for primary CMV infection in CMV D−/R− solid organ transplant recipients. Use of CMV-seronegative or leuko-reduced blood products for transfusion among transplant recipients has reduced the incidence of transfusion-transmitted CMV infection [35].

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25.2.2 Reactivation (CMV D−/R+)

This mechanism occurs when endogenous latent CMV in a CMV-seropositive (CMV R+) transplant recipient reactivates during the periods of impaired immunity after solid organ transplantation. Because CMV R+ patients have preexisting CMV-specific cell-mediated and humoral immunity, the degree of CMV replication is lower compared to primary CMV infection in CMV D+/R− patients and hence a relatively lower risk of CMV disease [36, 37].

25.2.3 Superinfection (CMV D+/R+)

Superinfection (also termed reinfection) occurs when a CMV R+ transplant recipient is infected with an exogenous CMV strain from a CMV-seropositive donor (or other exogenous sources such as blood transfusion), and subsequently, either the donor allograft-transmitted exogenous CMV or recipient-derived endogenous CMV, or both, reactivate to cause clinical disease [38]. In the majority of cases, donor-derived CMV strain is the predominant virus that reactivates in CMV D+/R+ patients [38–40]. However, recent analysis of the CMV genomic variants indicates complex reactivation patterns after solid organ transplantation, and often, multiple viral strains reactivate concurrently, making it difficult to distinguish donor or recipient origin [30, 31].

25.3 Mechanisms of Cytomegalovirus Reactivation After Solid Organ Transplantation

Table 25-1 lists the major risk factors for CMV infection and disease after solid organ transplantation [27]. Central to these factors is a pro-inflammatory state characterized by secretion of cytokines [41–43], most notably TNF-α, which is a potent CMV transactivator [44]. Stimulation of the TNF-α1 receptor results in the activation of protein kinase C and nuclear factor-kappa B (NF-κB), which translocates into the nucleus and binds to the CMV-immediate early enhancer/promoter region [41, 45–48], triggering a cascade of events that lead to CMV reactivation [49]. Likewise, catecholamines that are released during periods of stress, whether physiologic or in response to critical illness, could stimulate CMV-immediate early enhancer/promoter activity through the cyclic adenosine monophosphate (cAMP) pathway [45, 46]. Collectively, inflammation, stress, and other factors that influence the cAMP and NF-κB signaling pathways could lead to CMV reactivation after solid organ transplantation. Indeed, conditions characterized by high TNF-α levels, such as bacterial sepsis and allograft rejection, have been associated with CMV infection in solid organ transplant and non-transplant settings [29, 50–52].

TABLE 25-1. Factors that influence the reactivation of CMV and the risk of clinical disease after solid organ transplantation

Factors that influence CMV reactivation	Factors that influence progression to CMV disease	
	Increase risk of CMV disease	Diminish risk of CMV disease
Allogeneic stimulation	Lack of CMV-specific immunity (i.e., D+/R−)	Preexisting CMV-specific immunity (R+)
Allograft rejection	CMV-seronegativity	CMV-seropositivity
Lymphocyte-depleting agents	Deficient CMV-specific T cells	CMV-specific CD4/CD8 T cells
ATG, ALG, OKT3	Pharmacologic immunosuppression	Antiviral prophylaxis
Stress	ATG, ALG, OKT3	Preemptive antiviral therapy
Critical illness	Alemtuzumab	Immunotherapy
Surgical procedure	Mycophenolate mofetil (>2 g/day)	Immunoglobulins
Bacterial sepsis	High-dose methylprednisolone	Adoptive T cell therapy[a]
Fungal sepsis	Viral burden (viral load)	CMV vaccine[a]
		Reduced immunosuppression
		mTOR inhibitors
		Sirolimus
		Everolimus

CMV cytomegalovirus, *OKT3* muromonab-CD3, *ATG* anti-thymocyte globulin, *ALG* antilymphocyte globulin, *D+/R−* donor positive/recipient negative, *R+* recipient positive.
[a]Investigational.

25.4 Risk Factors for CMV Infection

The incidence rates of CMV disease after solid organ transplantation vary, depending on several factors such as underlying immunity (or lack thereof), viral factors, and the type of transplant (Table 25-2) [53–55]. In general, these factors can be categorized into (a) those that favor disease progression (such as delayed immune recovery, immunosuppressive drugs, viral load, viral coinfections) and (b) those that control viral replication and prevent disease (such as CMV-specific immunity, antiviral therapy) (Table 25-1).

25.4.1 CMV D+/R− Serostatus

CMV D+/R− mismatch serostatus is the most common clinical predictor of CMV infection and disease after solid organ transplantation. Hence, it is standard practice to measure CMV-IgG (a measure of previous exposure and a surrogate marker for the presence of latent virus; see Laboratory Diagnosis of CMV Infection section below) on all transplant candidates and donors in order to stratify the risk of disease, and guide the type of CMV prevention strategy [56]. Historical data indicate that up to 70% of CMV D+/R− solid organ transplant recipients, who lack preexisting CMV-specific cellular and humoral immunity, will

TABLE 25-2. Estimated incidence rates of CMV disease after solid organ transplantation

	CMV D+/R−		CMV R+	
Type of solid organ transplant±	No preventive strategy	Prophylaxis[c]	No preventive strategy	Prophylaxis[c]
Kidney and/or pancreas	45–65%	6–29%	8–10%	1–2%
Liver	45–65%	6–29%	8–19%	4–13%
Heart	29–74%	19–30%	20–40%	2%
Lung and lung-heart	50–91%	36–40%[a] 4–10%[b]	35–59%	10%[a] <5%[b]

CMV cytomegalovirus, *D+/R−* donor positive, recipient negative, *R+* recipient positive.
±The estimated incidence after small bowel transplantation is 22% of all patients, including all donor and recipient CMV serostatus.
[a]Antiviral prophylaxis is given for a duration of 3 months unless otherwise indicated ([b] indicates 6–12 months of prophylaxis after lung transplantation).
[c]CMV disease in patients who received prophylaxis generally occurs after the completion of antiviral prophylaxis (delayed-onset CMV disease).

develop primary CMV infection and disease after transplantation, if they do not receive any CMV prevention strategy (Table 25-2) [27]. The lack of CMV-specific immunity in CMV D+/R− solid organ transplant recipients allows for a very rapid CMV replication dynamics, with an estimated CMV doubling rate of 1 day [57]; this translates to high rates of CMV disease [36, 37]. Mathematical estimates suggest that CMV doubling rate is slower among solid organ transplant patients with adequate lymphocyte function [58]. Indeed, preexisting immunity in CMV R+ solid organ transplant recipients is believed to dampen CMV replication dynamics [36, 37]. Hence, reactivation and reinfection are characterized by a slower CMV replication dynamics, lower viral load, and lower incidence and severity of CMV disease in CMV R+ solid organ transplant patients [36, 37, 59, 60].

25.4.2 Defects in CMV-Specific T-Cell Immunity

CMV-specific T lymphocytes are essential for the adequate control of infection [59–62]. Expectedly, CMV-specific CD4+ and CD8+ T lymphocytes are absent in CMV D+/R− mismatch patients, thereby predisposing to high levels of viral replication and greater severity of CMV disease [61, 63, 64]. In contrast, preexisting CMV-specific CD4+ and CD8+ T lymphocytes control CMV infection in CMV R+ solid organ transplant recipients, although the lymphocyte population and function can be severely impaired by immunosuppressive drugs [61, 62, 64, 65]. In this context, intense pharmacologic immunosuppression is a major risk factor for CMV disease in CMV D+/R− and R+ solid organ transplant recipients. Studies have attempted to estimate the optimal threshold of CMV-specific CD4+ and CD8+ T lymphocytes sufficient for protection from CMV disease, but these values have not been precisely defined (see Laboratory Diagnosis of CMV Infection section below) [61, 62, 64–66].

25.4.3 Hypogammaglobulinemia

Serum IgG level <700 mg/dl is common during the first year after solid organ transplantation [67]. Its incidence is highest among lung (69%) and heart (48%) recipients and lowest among liver recipients (16%) [67]. Severe hypogammaglobulinemia, defined as IgG level <400 mg/dl, has been associated with a higher risk of opportunistic infections, including CMV [67]. A small study reported that CMV-hyperimmunoglobulin replacement was associated with low incidence of CMV disease in heart recipients with hypogammaglobulinemia [68]. However, a recent study demonstrated that intravenous immunoglobulin and CMV-hyperimmunoglobulin supplementation did not improve overall patient and graft survival outcomes [69].

25.4.4 Defects in Innate Immunity

Deficiencies in innate immunity have been implicated as risk factors for CMV disease after solid organ transplantation [70–76]. In some studies, defects in cell signaling through Toll-like receptors, mannose-binding lectin, mannose-associated serine protease-2, and activating and inhibitory killer cell immunoglobulin-like receptors have been significantly associated with a higher incidence of CMV disease after solid organ transplantation [8, 71–78].

25.4.5 Pharmacologic Immunosuppression

Induction therapy and maintenance immunosuppressive drugs, which are administered for the prevention and treatment of allograft rejection, significantly impair adaptive immune responses to CMV. These drugs, especially lymphocyte-depleting agents, dampen CMV-specific cell-mediated and humoral immune responses, thereby allowing for CMV disease development [52, 79]. Among the drugs most commonly associated with CMV disease are high-dose mycophenolate mofetil, anti-lymphocyte globulins (ALG),

anti-thymocyte globulins (ATG), alemtuzumab, and high-dose steroids [80–90]. Administration of lymphocyte-depleting antibodies is often complicated by a cytokine release syndrome, characterized by high levels of TNF-α, fevers, and rigors [91], which could theoretically induce CMV reactivation [80]. In addition to the individual effects of specific immunosuppressive drugs, the net state of immunosuppression is a major factor predisposing to CMV disease after solid organ transplantation. In contrast, the mTOR inhibitors (everolimus and sirolimus) appear to be associated with a lower incidence of CMV disease after solid organ transplantation [92–94].

25.4.6 Allograft Rejection

Acute rejection is one of the major risk factors for CMV disease after solid organ transplantation [52, 79, 95–97]. Pro-inflammatory cytokines, such as TNF-α, released during acute rejection are a potent trans-activator of CMV. In one study, acute rejection increased the risk of CMV disease by sixfold in a cohort of CMV D+/R− liver and kidney recipients [52]. Conversely, CMV increases the risk of acute and chronic rejection, thereby establishing their bidirectional "synergistic" relationship after solid organ transplantation [79, 98]. Antiviral prophylaxis has been associated in some studies to reduce not only the incidence of CMV disease but also acute rejection [32, 99, 100].

25.4.7 Type of Organ Transplant

Lung, small intestinal, and pancreas recipients generally have the highest risk, while liver and heart recipients have intermediate risk, and kidney recipients have the lowest risk for CMV disease [27, 56, 101]. Composite tissue allograft transplant recipients are also at high risk of CMV disease [102, 103]. While this heightened risk for some types of transplant may be due to high intensity of drug-induced immunosuppression, it has also been suggested that the large amount of latent CMV harbored in the allograft (i.e., viral burden) may contribute to the increased predisposition of lung and small intestinal recipients to develop CMV disease [27, 101].

25.4.8 Viral Burden and Genotype

The degree of viremia, as measured by nucleic acid testing and antigenemia, is a predictor of CMV disease after solid organ transplantation [36, 37, 104–108]. Genetic variability of viral strains (i.e., the viral genotype) has also been suggested to influence the risk of CMV [109], potentially accounting for the higher rate of CMV disease among CMV D+/R+ compared to CMV D−/R+ patients [30, 31]. The majority of reactivated CMV strains in CMV D+/R+ solid organ transplant recipients appear to be of donor origin [38–40].

25.4.9 Bacterial, Fungal, and Other Viral Infections

Occurrence of bacterial and fungal infections has been associated with a subsequent higher incidence of CMV disease after solid organ transplantation [29, 110–112]. These pro-inflammatory conditions would favor CMV reactivation, and lead to clinical disease. Conversely, CMV increases the risk of other opportunistic infections, possibly through CMV-induced immunomodulation [112–114]. CMV disease is more common in liver and kidney recipients with human herpes virus (HHV)-6 and HHV-7 infections [96, 115–119]. The underlying mechanism for the association is not defined, although HHV-6 and HHV-7 have immunomodulating properties that could predispose to CMV disease [116, 117, 119, 120]. Alternatively, the heightened predisposition to develop other opportunistic infections in patients with CMV disease may merely indicate a severely impaired global immune function.

25.5 Clinical Manifestations of CMV Disease

CMV infection in solid organ transplant recipients exhibits a wide spectrum of clinical symptoms [121, 122]. Based on these clinical manifestations, CMV infection may be classified either as an asymptomatic infection (termed subclinical CMV infection) or symptomatic infection (termed CMV disease). CMV disease can be further classified based on the presence of organ involvement (termed tissue-invasive CMV disease) or lack thereof (termed CMV syndrome) (Table 25-3) [56, 123, 124].

Traditionally, the onset of CMV infection and disease occur most commonly during the first 3–4 months after solid organ transplantation [80, 125–128]. However, the onset has been delayed in some populations, especially among CMV D+/R− solid organ transplant recipients who are receiving anti-CMV prophylaxis; in these patients, CMV infection occurs most commonly during the first 3–6 months after completion of prophylaxis [28, 29, 32, 129–135].

25.5.1 Direct CMV Effects

CMV disease is characterized by the reactivation of the virus in latently infected cells (including the allograft), dissemination in the blood, and invasion and replication in target organs (such as the allograft and other organs, most commonly the gastrointestinal tract). Clinically, this produces a characteristic febrile illness, often accompanied by bone marrow suppression, and various end-organ invasive diseases such as pneumonitis, gastritis, enteritis, colitis, encephalitis, hepatitis, retinitis, among others (Table 25-3). The

TABLE 25-3. Impact of CMV on solid organ transplantation

Direct effects	Indirect effects	Other associated outcomes
CMV syndrome	Acute allograft rejection	Increased total cost
Tissue-invasive CMV disease	Chronic rejection and allograft failure	Increased resource utilization
Gastrointestinal disease	Bronchiolitis obliterans (lung)	Prolonged hospitalization
Hepatitis	Cardiac vasculopathy (heart)	
Pneumonitis	Tubulointerstitial fibrosis (kidney)	
CNS disease	Opportunistic and other infections	
Retinitis	Fungi (*Aspergillus*, *Pneumocystis*)	
Nephritis	Bacteria (i.e., *Nocardia*)	
Pancreatitis	Epstein–Barr virus-associated PTLD	
Carditis	Hepatitis C recurrence	
Others[a]	Other viruses (HHV-6, HHV-7)	
Mortality (direct)	New-onset diabetes mellitus	
	Mortality (indirect and all-cause)	

PTLD posttransplant lymphoproliferative disease, *HHV* human herpes virus.
[a]Any organ system may be affected by cytomegalovirus.

diagnosis of CMV disease is confirmed by demonstrating the virus in the blood, other body fluids, or tissue specimens [121, 124]. Table 25-4 lists the various laboratory methods used for the diagnosis of CMV infection and disease after solid organ transplantation [136].

25.5.1.1 *CMV Syndrome*

CMV syndrome is the most common clinical presentation of CMV disease, and accounts for over 60–90% of all cases, after solid organ transplantation [28, 29, 124, 133, 137, 138]. CMV syndrome is the term for CMV disease without end-organ involvement, and it is most commonly manifested as fever (>38 °C for at least 2 days) and constitutional symptoms such as new or increasing malaise [121, 124, 139]. CMV syndrome is also characterized by bone marrow suppression, with patients presenting most commonly with new or increasing leukopenia and thrombocytopenia [121, 124, 139]. In some cases, atypical lymphocytosis and elevation in hepatic enzymes may be observed [121, 139]. In all cases of CMV syndrome, CMV should be demonstrated in the blood, either by nucleic acid testing or pp65 antigenemia [121, 124, 139]. For definite diagnosis of CMV syndrome, there should be no other etiology that could account for the clinical symptoms, such as bacterial and other viral infections [121, 139]. In some cases, however, coinfections with HHV-6 and HHV-7 may be demonstrated, although the role of these viruses in the clinical manifestations of CMV syndrome is debated [140–144].

25.5.1.2 *Tissue-Invasive CMV Disease*

CMV disease may manifest with signs and symptoms of organ dysfunction [27–29, 32, 121, 132, 133, 139, 145]. Patients with organ-specific symptoms should be suspected as having tissue-invasive CMV disease, and the diagnosis confirmed by the demonstration of CMV by histopathology (i.e., inclusion bodies and cytopathic changes), immunohistochemistry, or in situ DNA hybridization [121, 124, 139]. In most cases of tissue-invasive CMV disease, the virus is also demonstrated in the peripheral blood by nucleic acid testing or pp65 antigenemia. However, there is a proportion of localized or compartmentalized tissue-invasive CMV disease with no detectable virus in the blood [27]; in these cases, a tissue biopsy is needed to confirm the diagnosis of tissue-invasive disease [27, 121, 139].

The most common form of tissue-invasive disease is gastrointestinal CMV disease, which can involve any part of the gastrointestinal tract [27–29, 32, 132, 133, 145–147]. The most severe forms of tissue-invasive CMV disease are pneumonia and central nervous system disease (e.g., encephalitis) [129]. In some cases, multiple organs may be involved, such as concomitant CMV colitis and hepatitis. It is rare for solid organ transplant recipients to develop CMV retinitis, although this diagnosis should be considered and screened for in patients with compatible symptoms [101, 148]. Virtually any organ system can be affected by CMV, and some of the less common sites of tissue involvement are the gallbladder and biliary tree, epididymis, skin, and endometrium [149–154]. Congenital CMV has been reported in the offspring of female transplant recipients [155, 156].

CMV disease may involve the transplanted allograft, especially among CMV D+/R− patients. This is not unexpected since the virus is harbored in the allograft. CMV reactivates in the transplanted organ and cause graft dysfunction and allograft tissue-invasive CMV disease [157–161]. Hence, liver recipients may present with CMV hepatitis [162], pancreas recipients with CMV pancreatitis [157], kidney recipients with CMV nephritis [163], heart recipients with CMV myocarditis [161], and lung recipients with CMV pneumonitis [158–160]. Because the clinical manifestations of tissue-invasive CMV disease are difficult to differentiate from allograft rejection, tissue biopsy for definitive diagnosis is imperative to guide appropriate therapy [121, 139].

The clinical manifestations of tissue-invasive CMV disease depend on the organ and severity of involvement. CMV hepatitis typically manifests with fever and elevated serum levels of gamma-glutamyl transferase, alkaline phosphatase, and aminotransferases, with minimal elevations in serum bilirubin levels [162]. CMV pneumonitis manifests as fever, dyspnea, and cough, accompanied by hypoxemia and radiographic findings of bilateral interstitial, unilateral lobar, or nodular pulmonary infiltrates [164]. CMV can affect any segment of the gastrointestinal tract [165], and depending on

TABLE 25-4. Laboratory methods for the diagnosis of CMV infection and disease after solid organ transplantation

Method	Examples	Principle	Clinical use	Comments
Viral culture	Tube culture Shell vial assay	Virus isolation	Diagnosis of CMV infection and disease	High specificity but poor sensitivity Slow turnaround time Virus isolate may be used for phenotypic drug susceptibility
Serology	ELISA	Antibody detection (IgG, IgM)	Pretransplant assessment of CMV exposure (IgG only) Not used for real-time diagnosis of acute disease after transplantation	IgG preferred for screening IgM suggests acute infection, but caution for false positive results
Antigenemia	Slide method	pp65 antigen detection	Rapid diagnosis of CMV infection and disease Guide for preemptive therapy Guide for duration of antiviral therapy	More sensitive than shell vial assay Reduced sensitivity during leukopenia and neutropenia Labor intensive and lacks standardization Operator-dependent (subjective interpretation) Requires immediate processing (relies on life span of leukocytes ex vivo)
Nucleic acid tests	Commercial assays Laboratory-developed assays	Viral nucleic acid (DNA or RNA) detection	Rapid diagnosis of CMV infection and disease Assessment of risk of CMV disease Guide for initiation of preemptive therapy Guide for duration of antiviral therapy Marker for risk of relapse and drug resistance	Quantitation allows for assessing severity of infection; qualitative assay does not distinguish latency from active replication Standardization with the use of calibrators Thresholds for various clinical indications have not been fully determined to be applied widely

ELISA enzyme linked immunosorbent assay, *CMV* cytomegalovirus.

the segment involved, it may manifest with dysphagia and odynophagia (CMV esophagitis), nausea, vomiting, and abdominal pain (CMV gastritis), gastrointestinal hemorrhage (CMV gastritis, enteritis, or colitis), and diarrhea (CMV enteritis and colitis) [166–168]. The endoscopic findings of gastrointestinal CMV disease may be nonspecific such as erythema and diffuse, shallow erosions, but characteristic ulcers are typically observed [167]. Histopathologic examination to demonstrate cytomegalic cells with CMV inclusion bodies in tissue specimens, the use of in situ hybridization or CMV-specific immunochemical stains to demonstrate the presence of the virus in biopsy specimens are necessary to confirm tissue-invasive CMV disease [166]. CMV has been reported to cause vasculitis that resulted in ischemic colitis [169], or hepatic artery thrombosis [170, 171]. Rarely, CMV may cause retinitis, usually at a later stage beyond 6 months after solid organ transplantation [101]. Patients with CMV retinitis may be asymptomatic, or they may experience blurring of vision, scotomata, or decreased visual acuity [101]. Characteristic fundoscopic findings by an experienced ophthalmologist often reveal the diagnosis of CMV retinitis, although the demonstration of CMV in vitreous fluid by nucleic acid testing may be necessary in atypical cases [101]. Central nervous system involvement by CMV occurs very rarely, and it is manifested as change in mental function and confirmed by the demonstration of CMV by nucleic acid testing of the cerebrospinal fluid [153].

25.5.2 Indirect CMV Effects

CMV is associated with numerous indirect effects (Table 25-3), which occur as a result of its ability to modulate the immune system, either directly or through the upregulation of cytokines, chemokines, growth factors, and other immune molecules [172]. Some of the indirect effects may be delayed direct effects of persistent subclinical CMV infection in the transplanted allograft (e.g., chronic allograft failure) [173].

25.5.2.1 Opportunistic Infections

CMV causes immunomodulation that predisposes to opportunistic bacterial and fungal infections after solid organ transplantation [113, 174, 175]. Studies have shown that the incidence of bacterial and fungal infections was lower among solid organ transplant recipients who received antiviral prophylaxis [114, 176, 177]. The immunomodulatory property of CMV is postulated to increase the risk of Epstein–Barr virus-induced posttransplantation lymphoproliferative disorders (PTLD) in transplant patients [178].

25.5.2.2 Allograft Rejection

CMV has been associated with reduced allograft survival after liver, kidney, lung, pancreas, and heart transplantation [29, 179, 180]. Acute rejection was significantly higher

among CMV-infected transplant recipients [181]. Prolonged CMV replication has been associated with an increased risk of chronic rejection after liver transplantation [182]. Although the mechanisms underlying these associations have not been fully elucidated, persistent CMV replication may mediate persistent T-cell stimulation, either directly or indirectly, by increasing the immunogenicity of the allograft (i.e., γ-interferon-mediated up-regulation of major histocompatibility complex antigens) resulting in a chronic inflammatory process [183].

25.5.2.3 Chronic Allograft Failure

CMV has been significantly associated with bronchiolitis obliterans syndrome, a form of chronic lung allograft rejection characterized by bronchiolar inflammation and granulation [160]. Ganciclovir prophylaxis has been reported to reduce the incidence of bronchiolitis obliterans after lung transplantation [184]. Among heart recipients, CMV has been implicated in the pathogenesis of accelerated vasculopathy [184–186]. CMV disease has been implicated as a factor for the development of vanishing bile duct syndrome, characterized by ductopenia, severe jaundice, and pruritus after liver transplantation [187–189]. Among kidney recipients, CMV has been associated with tubulointerstitial fibrosis and glomerulopathy, characterized by enlargement and necrosis of endothelial cells and accumulation of mononuclear cell infiltration and fibrillary material deposition in glomerular capillaries [29, 154].

25.5.2.4 Vasculopathy and Procoagulation

A significant association between CMV and vasculopathy has been reported after heart transplantation [185, 186, 190–192]. This association is supported by experimental data showing the ability of CMV to infect endothelial cells, influence smooth muscle cell migration and growth in vitro, and induce neointimal proliferation in rat aortic allografts [193]. Experimental in vivo data demonstrate that rat CMV causes endothelial inflammation that results in intimal thickening in aortic and cardiac allografts, and this was diminished by ganciclovir [194, 195] or experimental CMV vaccination [196]. In addition, CMV-enhanced allograft vasculopathy may be mediated by a proliferative effect on the smooth muscle cells and/or inflammatory cells with enhanced production of growth factors [190, 194, 195, 197, 198]. CMV infection of endothelial cells may also lead to a procoagulant response that could account for the clinical association between CMV and vascular thrombosis [171, 199, 200]. Antiviral drugs together with CMV-hyperimmune globulins have reduced the risk of vasculopathy after heart transplantation [185, 186].

25.5.2.5 Viral Interactions

Reactivation of multiple latent viruses, including CMV, is common after solid organ transplantation, thereby creating an environment that would allow potential interactions [119, 141–143]. Such interaction may lead to altered clinical presentation of various viral infections [119, 120, 141–143, 179, 201, 202]. Examples are the proposed interactions among β-herpesviruses, which may be manifested as an increased incidence and severity of CMV disease [119, 120], the proposed association between CMV and EBV-associated PTLD [178], and the ability of CMV to accelerate the clinical course of recurrent hepatitis C, resulting in higher rates of inflammation, fibrosis, allograft failure and mortality after liver transplantation [201–203].

25.5.2.6 New-Onset Diabetes Mellitus After Transplantation

CMV has been associated with new-onset diabetes mellitus after transplantation. A meta-analysis of seven clinical trials reported that CMV was a risk factor for new onset diabetes mellitus [204]. However, the mechanism underlying this relationship is yet to be determined.

25.5.3 Mortality

Solid organ transplant recipients with CMV disease have a significantly higher mortality rate compared to those without CMV disease [29, 134, 205–208]. The causes of death are most commonly non-CMV related, although occasionally, CMV may directly cause death if not diagnosed and treated appropriately. The use of effective antiviral drugs for prevention and treatment has reduced all-cause mortality after solid organ transplantation [95, 111, 205, 209–212].

25.6 Laboratory Diagnosis of CMV Infection

The laboratory methods for the diagnosis of CMV infection detect (1) the virus in clinical samples, and (2) the immune response to the virus [104] (Table 25-4). The most common method to confirm clinical suspicion of CMV infection is a molecular test that quantifies viral nucleic acid in blood and other clinical samples [104]. Other methods that may be used for this purpose are pp65 antigenemia and viral culture, although their use has markedly declined during the last decade [104]. The diagnosis of tissue-invasive CMV disease is confirmed by histopathologic examination of biopsy samples. Serology to detect CMV IgG antibodies is the standard test to screen for prior exposure, and serve as a surrogate for

latent CMV, among donors and candidates [121]. A novel immunologic test undergoing clinical evaluation is the detection, quantification, and functional assessment of CMV-specific T lymphocytes [66].

25.6.1 Nucleic Acid Tests

Nucleic acid tests are the most common methods for the detection of CMV in blood and other clinical samples [104]. These tests are most commonly performed using polymerase chain reaction (PCR), which amplifies CMV nucleic acid in clinical samples of patients with active CMV infection [104]. There are numerous nucleic acid tests, including commercial and laboratory-developed (also known as "home-brew") tests. Until recently, the tests were not standardized, resulting in wide inter-assay variability in viral load reporting [213]. In a large multicenter study, which included 33 laboratories that were asked quantify the viral load of samples with known viral quantity, there was wide inter-laboratory variability in viral load values and limit of detection [213]. This variability in viral load values has limited direct comparison of practices among transplant centers. The calibration standard was identified as the most significant variable that accounted for the wide inter-assay variability [214, 215]. Hence, a WHO International Standard (National Institute for Biological Standards and Control, UK) and a certified reference material (National Institutes of Standards and Technology, USA) were developed [104]. Several commercial and laboratory-developed tests have now been optimized based on these calibration standards [104, 137, 216, 217].

Nucleic acid tests are used for several clinical indications, including (1) sensitive and rapid diagnosis of CMV infection, (2) assessment of the risk of CMV disease and its severity, (3) guiding initiation of preemptive therapy for preventing CMV disease, (4) assessment of the efficacy of antiviral therapy, (5) guiding the duration of antiviral treatment, and (6) assess the risk of relapse of infection and disease (Table 25-4) [104]. Three different viral load measures are often used for these clinical uses – absolute viral load, change in viral load over time, and viral load suppression. In one study, viral load of 3983 IU/ml was suggested and validated as the threshold for initiating preemptive therapy in CMV R+ solid organ transplant recipients [218]. In another study, a viral load threshold of 2275 IU/ml was suggested to discriminate self-resolving infection from those that need antiviral treatment in a cohort of CMV R+ solid organ transplant recipients [219]. A level of 2000 copies/ml was defined in another study as optimal threshold to initiate preemptive therapy in moderate risk CMV R+ kidney transplant recipients [220]. A lower viral load value may be more significant in CMV D+/R− solid organ transplant recipients, where clinical disease have been observed in as low as 1500 copies/ml, hence the need to treat high-risk patients even at much lower viral load values. In general, however, viral load values during the onset of CMV disease are significantly higher in CMV D+/R− compared to CMV R+ solid organ transplant recipients, and in tissue-invasive disease (mean value, 20,893 IU/ml) compared to CMV syndrome (9120 IU/ml) [137].

Nucleic acid tests have been compared, on multiple occasions, with antigenemia to compare their performance in diagnosis of CMV infection. While the results of numerous studies have been variable, there were more studies that indicated nucleic acid tests are more sensitive than pp65 antigenemia [104]. Indeed, more centers are currently relying on nucleic acid tests for diagnosis of CMV in solid organ transplant recipients.

25.6.2 Antigenemia

The pp65 antigenemia test is another common method for the diagnosis of CMV infection after solid organ transplantation, although its use is currently declining due to more widespread application of nucleic acid tests [219, 220]. The antigenemia test is based on the principle of detecting pp65 antigen that is expressed in polymorphonuclear cells during active CMV infection. Blood is collected and the polymorphonuclear cells are separated and stained with monoclonal antibody against the pp65 antigen. The number of pp65-staining polymorphonuclear cells are counted, and expressed per a predefined number of polymorphonuclear cells (for example, per 100,000 polymorphonuclear cells). Like nucleic acid tests, the pp65 antigenemia has been used for rapid diagnosis of CMV infection (more rapid than viral culture), assess severity of infection (higher number of cells suggest greater severity), guide preemptive therapy, and guide duration of antiviral therapy. Various cutoffs (e.g., number of positive cells) have been defined by different centers, and the variable thresholds identified are likely influenced by the underlying net state of immunity [219, 220].

25.6.3 Viral Culture

Detection of CMV using conventional culture methods and shell vial techniques have become less common due to their labor intensive nature, slow turnaround time, and low to modest sensitivity [104, 221–224]. While viral culture is highly specific for CMV disease, the sensitivity for viral detection is low. Moreover, the slow turnaround time limits real-time clinical applicability. Rapid and real-time tests such as nucleic acid tests have generally supplanted the use of viral culture in the clinical setting [104, 221].

25.6.4 Histopathology

The diagnosis of tissue-invasive CMV disease relies on tissue biopsy demonstrating histologic changes, including cytomegalic inclusion cells [121, 163]. In situ hybridization

and immunohistochemistry staining may be needed when the histologic findings are nonspecific. The most common organs biopsied are the gastrointestinal tract (the most common organ involved in tissue-invasive disease) and the transplanted allograft [163]. Allograft biopsy is often needed to distinguish CMV disease from allograft rejection [121]. One study suggested that detection of virus in the blood by nucleic acid testing may obviate the need for biopsy in patients presenting with symptoms of gastrointestinal CMV disease [147]. The overall sensitivity of this approach is 85%, which increases to 100% with primary gastrointestinal CMV disease but declines to 73% among CMV R+ solid organ transplant recipients [147]. The decline in sensitivity is accounted for by CMV R+ solid organ transplant recipients with compartmentalized or localized gastrointestinal CMV disease with low or absent viremia [147, 225]. Hence, biopsy should be considered when there is clinical concern for CMV disease even if the nucleic acid test in the blood is negative [121]. The virus is detected in the affected tissue for longer period compared blood [146]. In a study of primary gastrointestinal CMV disease, the virus was demonstrated in the tissue for at least 1 week beyond viral eradication in the blood [146]. However, it is not necessary to repeat endoscopy and intestinal biopsy to document clearance of infection before discontinuing antiviral treatment, except in cases of severe disease that involves multiple gastrointestinal segments [146].

25.6.5 Serology

CMV IgG serology is the standard test to screen transplant candidates and potential donors for previous exposure to CMV [121]; this serves as a surrogate marker of latent CMV. Based on the serologic testing of the donor and recipients, the risk of CMV infection and disease can be stratified as high risk (CMV D+/R−), moderate risk (CMV R+) and low risk (CMV D−/R−) [121]. Such risk profile assessment determines the type of CMV prevention strategy after solid organ transplantation [121]. One study further stratified the risk of CMV infection after liver transplantation among CMV R+ recipients based on the serologic titer; in this study, CMV R+ liver recipients with lower CMV-IgG titer has a higher risk of developing CMV disease [110]. However, serology should not be used to diagnose active CMV infection after transplantation, since patients have impaired ability to mount an effective immune response, resulting in delayed antibody production [121].

25.6.6 Cellular Immune Monitoring

Assessment of CMV-specific T cell number and function is an emerging tool to assess the risk of CMV after solid organ transplantation [66]. A variety of methods are available including cytokine flow cytometry, ELISpot, or assessment of cytokine secretion such as interferon-gamma (e.g., interferon-gamma release assays) [66]. Detection of functional CMV-specific T cells reflects the ability of a patient to mount an effective immune response, and a lower risk of CMV disease. Various thresholds for risk prediction have been suggested using different assays and patient populations, but their lack of standardization has limited their widespread clinical application. A positive QuantiFERON-CMV test, an investigational assay that measures interferon-gamma release after stimulation with CMV antigens ex vivo, was associated with lower risk of CMV disease in a large cohort of solid organ transplant recipients, including CMV D+/R− group [226, 227]. Several studies have examined the role of ELISpot, with variable positive thresholds as indicative of risk of CMV disease [228–230]. There are various studies using cytokine flow cytometry, with inconsistent results likely due to differences in techniques, reagents and antigens, patient populations and degree of immunosuppression [65, 66, 231, 232].

25.7 Prevention of Cytomegalovirus Infection and Disease

Because of the negative impact of CMV on outcomes, its prevention is a major focus of management after solid organ transplantation. The most common method is the use of antiviral drugs either as antiviral prophylaxis or preemptive therapy. In addition, a common practice is the use of CMV-seronegative, filtered, or leukocyte-reduced blood products when blood transfusion is necessary for patient care. CMV-seronegative donor and recipient matching, which would pair a CMV-seronegative donor and recipient, is logistically difficult and rarely performed. Waiting for a CMV-seronegative donor may result in an unnecessarily prolonged waiting time for a patient who needs a lifesaving transplant procedure. Currently, non-selected or CMV-hyperimmunoglobulin are less commonly used, partly because of its expense, modest efficacy, and the availability of effective antiviral drug strategies. There is no vaccine that is available clinically for active immunization against CMV in humans, although some are in early clinical development.

25.7.1 Antiviral Strategies

The two major antiviral strategies for preventing CMV disease after solid organ transplantation are (1) antiviral prophylaxis and (2) preemptive therapy [26, 56, 123, 233–237]. Antiviral prophylaxis entails the administration of an antiviral drug for a fixed duration of time (usually, a minimum of 3–6 months) to all patients or to “at-risk” patients after solid organ transplantation (Figure 25-1a) [56, 123, 233]. A variant of this approach is targeted prophylaxis, which administers antiviral drugs only during periods highly associated with CMV reactivation, such as those times when ATG,

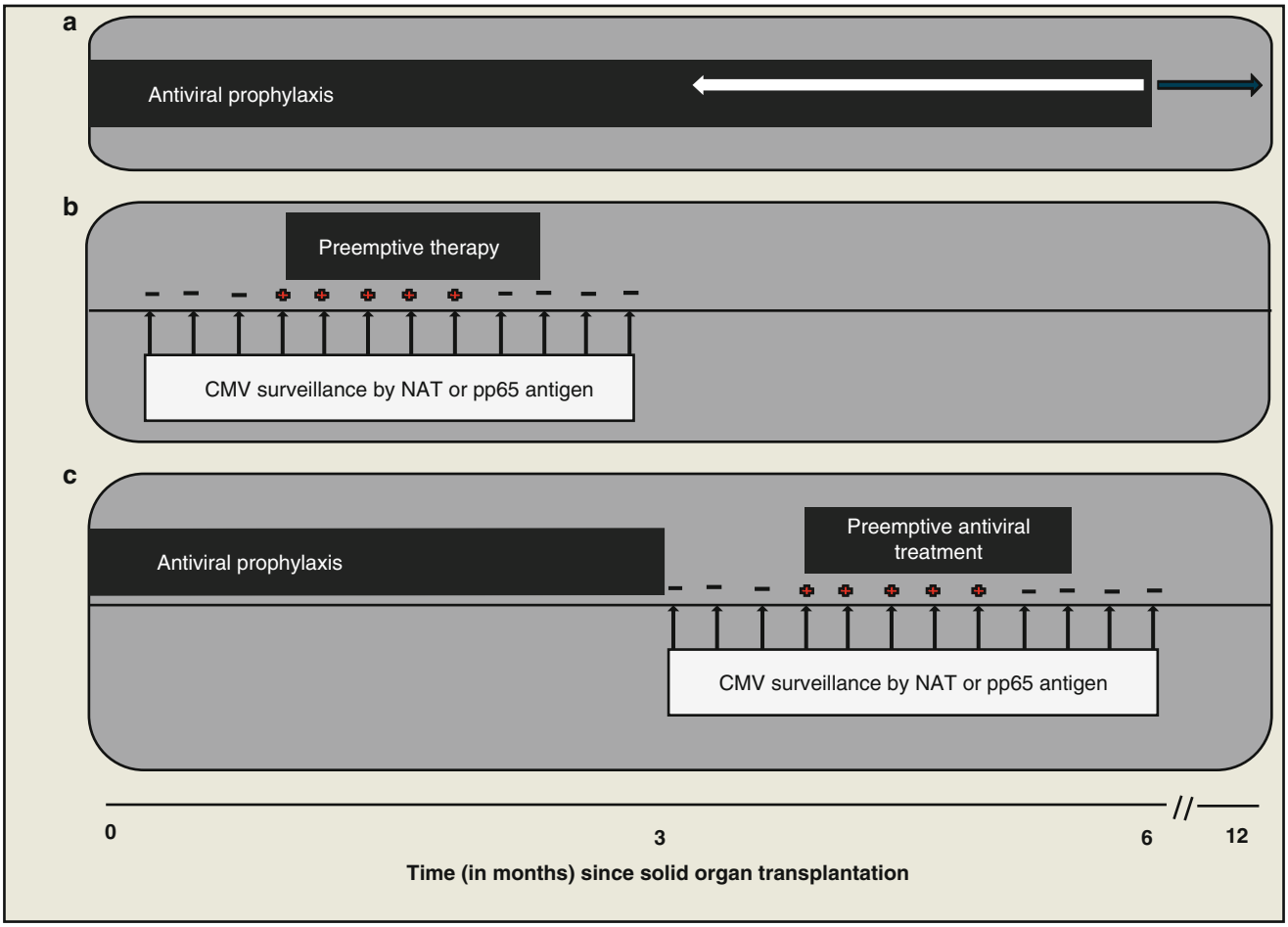

FIGURE 25-1. Schematic representation of antiviral prophylaxis (**a**), preemptive therapy (**b**) and a hybrid approach (**c**) after solid organ transplantation.

ALG, OKT3, and alemtuzumab are used for treatment of acute rejection [56, 123, 233]. In contrast, preemptive therapy involves the administration of antiviral drug only to transplant recipients with evidence of early asymptomatic CMV replication (Figure 25-1b) [56, 123, 233]. The goal of preemptive therapy is to treat early CMV reactivation prior to the onset of clinical disease [121].

There is an ongoing debate whether antiviral prophylaxis or preemptive therapy is the optimal strategy for preventing CMV disease after solid organ transplantation. It is generally believed however that both strategies are effective for CMV disease prevention [121, 238–240]. Several meta-analyses concluded that antiviral prophylaxis and preemptive therapy are both effective in preventing CMV disease (Table 25-5) [209–212]. A reduction in incidence of indirect effects was evident with antiviral prophylaxis [209–212], specifically, a reduction in all-cause mortality [27, 176, 241]. In head-to-head clinical trials that directly compared both strategies in cohorts of kidney recipients, both were similarly effective in preventing CMV disease (Table 25-6) [238–240]. The overall cost of both antiviral strategies appears to be similar, with cost of drug (for antiviral prophylaxis) being counterbalanced by the cost of laboratory monitoring (for preemptive therapy) [240]. Table 25-7 summarizes the potential benefits and disadvantages of the two antiviral strategies [27]. Which of these two strategies is more effective in a specific transplant setting or a specific transplant population is most likely influenced by a variety of factors, such as recipient and donor characteristics, underlying medical comorbidity, the logistics of frequent CMV surveillance, the availability of sensitive and rapid methods for CMV detection, and the availability and cost of antiviral drugs [242, 243]. Hence, the choice of which antiviral approach to use after solid organ transplantation is institution-, organ transplant-, and resource-dependent.

25.7.1.1 Antiviral Prophylaxis

The antiviral drugs for preventing CMV disease after solid organ transplantation are valganciclovir, oral and intravenous ganciclovir, and valacyclovir (Table 25-8) [32, 132, 135, 244–248]. Foscarnet and cidofovir are not routinely

TABLE 25-5. Meta-analyses of randomized controlled trials of antiviral prophylaxis and preemptive therapy for the prevention of cytomegalovirus disease after solid organ transplantation

Author (reference)	Study characteristics	CMV disease[a]	CMV infection[a]	All-cause mortality[a]	Other effects
Antiviral prophylaxis					
Hodson [241]	RCT of GCV, ACV, and VACV prophylaxis vs. placebo or no treatment	0.42 (0.34–0.52) 19 Trials 1981 Patients	0.61 (0.48–0.77) 17 Trials 1786 Patients	0.63 (0.43–0.92) 17 Trials 1838 Patients	Reduction in HSV, VZV, bacterial, and protozoal infections No significant effect on fungal infection, acute rejection, and graft loss
Kalil [210]	Prophylaxis vs. placebo or no treatment	0.20 (0.13, 0.31) 11 Trials 1582 Patients	NA	0.62 (0.40–0.96) 7 Trials 1338 Patients	Reduction in allograft rejection (RR: 0.74; 95% CI: 0.59–0.94)
Small [211]	GCV prophylaxis vs. placebo or no treatment	0.34 (0.24–0.48) 8 Trials 930 Patients	NA	0.99 (0.68–1.43) 12 Trials 1322 Patients	No significant reduction in rejection (RR: 0.90; 95% CI: 0.79–1.01)
Hodson [339]	RCT of IgG vs. placebo or no treatment	0.80 (0.61–1.05) 16 Trials 770 Patients	0.94 (0.80–1.10) 14 Trials 775 Patients	0.57 (0.32–1.03) 8 Trials 502 Patients	Reduction in CMV-related deaths (RR: 0.33; 95% CI: 0.14–0.80) No significant difference in the risk of CMV disease, CMV infection, and all-cause mortality between antiviral drug combined with IgG compared to antiviral medication alone
Preemptive therapy					
Hodson [241]	RCT of preemptive therapy vs. placebo or no treatment	0.29 (0.11–0.80) 6 Trials 288 Patients	NA	1.23 (0.35–4.30) 2 Trials 176 Patients	No significant effect of acute rejection (RR: 1.06; 95% CI: 0.64–1.76)
Kalil [210]	Preemptive therapy vs. placebo or no treatment	0.28 (0.11–0.69) 6 Trials 398 Patients	NA	0.94 (0.32–2.76) 3 Trials 253 Patients	Significant reduction in allograft rejection (RR: 0.47; 95% CI: 0.24–0.91)
Small [211]	Preemptive therapy with GCV	0.30 (0.15–0.60) 9 Trials 457 Patients	NA	0.94 (0.43–2.07) 4 Trials 208 Patients	No significant reduction in rejection (RR: 0.54; 95% CI: 0.29–1.01)

RCT randomized controlled trial, *NA* not assessed, *RR* relative risk, *95% CI* confidence intervals, *IgG* immunoglobulin G, *HSV* herpes simplex virus, *VZV* varicella zoster virus, *CMV* cytomegalovirus, *GCV* ganciclovir, *ACV* acyclovir, *VACV* valacyclovir.
[a]Data presented as Relative Risk (95% confidence intervals), number of trials included, and number of patients.

TABLE 25-6. Selected clinical trials comparing preemptive therapy and antiviral prophylaxis in kidney transplant recipients

Study reference	Trial A [240]	Trial B [238]	Trial C [239]
Total no. of patients	98	70	148
No. patients for preemptive therapy versus antiviral prophylaxis	49 vs. 49	36 vs. 34	74 vs. 74
% CMV D+/R−	33% vs. 26%	17% vs. 12%	33.8% vs. 30.1%
Monitoring strategy for preemptive therapy	qPCR WB weekly × 16 weeks	qPCR WB weekly × 16 weeks	qPCR WB weekly × 4 weeks then Q2 weeks until 12 weeks
Drug for preemptive therapy	VGCV 900-mg BID x21d	VGCV 900-mg BID x≥14d	IV GCV 5-mg/kg BID x≥10d
Drug for antiviral prophylaxis	VGCV 900-mg QD × 100d	VACV 2-g QID x90d	Oral GCV 1-g TID x90d
Outcomes (preemptive therapy versus antiviral prophylaxis)			
CMV infection	59% vs. 29% ($p=0.004$)	92% vs. 59% ($p<0.001$)	51% vs. 18% ($p<0.001$)
CMV disease	2% vs. 8% ($p=0.362$)	6% vs. 9% ($p=$ns)	18.5% vs. 6.8% ($p=0.04$)
Biopsy-proven allograft rejection at 12 months	8% vs. 2% ($p=0.36$)	36% vs. 15% ($p=0.034$)	28% vs. 19% ($p=$ns)
Mortality	0% vs. 0%	0% vs. 3% ($p=$ns)	5% vs. 7% ($p=$ns)
Other outcomes	No difference in overall cost	No difference in overall cost	Comparable CrCl at 12 mo Prophylaxis increased long-term survival (92% vs. 78%; $p=0.04$) CMV associated with severe impairment in graft function

CMV cytomegalovirus, *D+/R−* donor positive/recipient negative, *GCV* ganciclovir, *VGCV* valganciclovir, *VACV* valacyclovir, *d* days, *w* weeks, *mo* months, *CrCl* creatinine clearance.

TABLE 25-7. Benefits and risks of universal antiviral prophylaxis and preemptive therapy against CMV after solid organ transplantation

Strategy	Benefits	Risks and disadvantages
Universal prophylaxis	• Prevents reactivation of other herpes viruses (i.e., herpes simplex virus, HHV-6) • Does not rely on frequent laboratory monitoring for CMV detection • Reduces incidence of indirect CMV effects (acute rejection, chronic allograft failure, opportunistic bacterial and viral infections, posttransplant lymphoproliferative disease, mortality)	• Prolonged antiviral drug use may lead to the emergence of antiviral drug resistance • Prolonged antiviral drug use may lead to higher incidence of adverse drug effects • Associated with late-onset CMV disease
Preemptive therapy	• Reduced number of patients exposed to antiviral drugs • Reduced direct drug costs • Reduced duration of antiviral drug use • Reduced toxicity related to antiviral drugs • Lower risk of antiviral drug resistance (although resistance has been observed with prolonged preemptive therapy)	• Requires a predictive test for early identification of patients at risk of CMV disease • Requires patients to comply with stringent surveillance schedule • Requires personnel to actively implement the logistics of the CMV surveillance program • Increased cost of diagnostic surveillance testing • May not identify all patients at risk of CMV disease because of rapid viral replication in CMV D+/R− patients • CMV-selective nature does not prevent reactivation of other herpes viruses

CMV cytomegalovirus, *HHV* human herpesvirus.

TABLE 25-8. Selected randomized controlled clinical trials of antiviral prophylaxis after solid organ transplantation

Type of transplant	Reference	Study characteristics	Number of patients per group (# of D+/R− patients[a])	Prophylactic regimen	Outcome and comments	CMV disease[b]	Mortality[b]
Valganciclovir vs. *oral ganciclovir*							
Kidney, pancreas, liver, and heart	[135]	Primary prophylaxis; prospective, randomized, controlled, multicenter	245 (245) vs. 127 (127)	VGCV 900-mg once daily vs. GCV 1-g PO TID	Similar overall incidence of CMV disease Higher rate of tissue-invasive CMV disease in liver recipients on VGCV vs. oral GCV prophylaxis Higher incidence of neutropenia in VGCV group	12.1% vs. 15.2% at 6 months 17.2% vs. 18.4% at 12 months Investigator-treated CMV: 30.5% vs. 28%	Not reported
Kidney	[131]	Primary prophylaxis; randomized controlled multicenter trial	All D+/R− 326 (326)	VGCV 900-mg once daily for 200 days vs. 100 days	Lower incidence of CMV viremia and disease with 200 days of prophylaxis compared to 100 days of prophylaxis	16.1% vs. 36.8% at 12 months	No deaths in 200 day group compared to 4 deaths in the 100 day group
Lungs	[129]	Primary prophylaxis; randomized controlled multicenter trial	66 (25) vs. 70 (20)	VGCV 900-mg once daily for 3 months vs. 12 months	Significant reductions in CMV infection, and disease severity	32% vs. 4%	Not reported
Ganciclovir vs. *other regimens (including placebo)*							
Kidney	[247]	Primary prophylaxis	17 (17) vs. 17 (17)	IV GCV 5 mg /kg 2×/day × 14 days (days 14–28) vs. No ganciclovir	Delayed onset CMV infection; Reduced severity of CMV disease; no change in incidence of CMV infection or disease	47% vs. 73%	0% vs. 0%

(continued)

TABLE 25-8. (continued)

Type of transplant	Reference	Study characteristics	Number of patients per group (# of D+/R− patients[a])	Prophylactic regimen	Outcome and comments	CMV disease[b]	Mortality[b]
Heart	[246]	Prospective, double-blind	76 (19) vs. 73 (16)	IV GCV 5 mg/kg 2×/day × 14 days, then 6 mg/kg 5/week × 14 days vs. placebo	Reduced incidence of CMV disease in the R^+ subgroup Delayed incidence of CMV shedding	35% vs. 29% in D+/R− 9% vs. 46% in R+	4% vs. 1%
Lung	[245]		13 (3) vs. 12 (3)	IV GCV 5 mg/kg 4×/day × 2 weeks; then 5 mg/kg/day × 1 week; then 5 mg/kg/day for 5 days/week through 90 days vs. IV GCV 5 mg/kg 4×/day × 2 weeks; then 5 mg/kg/day × 1 week; then PO ACV 800 mg 4×/day through 90 days	Decreased CMV infection and disease; increased median infection-free duration	0% vs. 25%	15% vs.25%
Liver	[132]	Prospective; double-blind, placebo-controlled	150 (21) vs. 154 (25)	PO GCV 3 g/day × 98 days vs. placebo	Reduced CMV infection (24.5% vs. 51.5%); reduced HSV infection	4.8% vs. 18.9% (14.8% vs. 44% among D+/R−)	6.7% vs. 10.4%
Acyclovir or valacyclovir vs. *placebo or no prophylaxis*							
Kidney	[244]	Prospective, double-blind	53 (6) vs. 51 (7)	PO ACV 800 mg 4×/day × 12 weeks vs. placebo	Decreased CMV infection and disease, especially in the D^+/R^- subgroup	8% vs. 29%	4% vs. 6%
	[32]	Prospective	306 (102) vs. 310 (106)	PO VACV 2 g 4×/day × 90 days vs. placebo	Reduced allograft rejection and incidence of HSV Increased hallucinations and confusion	16% vs. 45% in D+/R−; 1% vs. 6% in R+	5% vs. 4% in D+/R− 1% vs. 5% in R+
Liver	[248]	Prospective	60 (0) vs. 60 (0)	IV ACV 500 mg/m^2 3×/day × 10 days, then PO ACV 3200 mg/day through 3 months vs. no prophylaxis	Decreased incidence of CMV infection and disease	7% vs. 23%	No effect on patient survival

CMV cytomegalovirus, *HSV* herpes simplex virus, *D* donor, *R* recipient, *PO* per orem (orally administered), *IV* intravenous, *ACV* acyclovir, *VACV* valacyclovir, *GCV* ganciclovir.

[a]If known, the number of patients who were D^+/R^- is given in parentheses.

[b]Amongst patients given, first listed vs. second listed regimen.

used for prevention of CMV disease after solid organ transplantation because of renal and other toxicities [27]. Numerous clinical trials have demonstrated the efficacy and safety of antiviral drugs for prevention of CMV disease after solid organ transplantation (Table 25-8) [32, 132, 135, 244–248]. As demonstrated in these clinical trials, the immediate benefits of antiviral prophylaxis are the reduction in the incidence and severity of CMV disease. In some trials, a

TABLE 25-9. Recommendations for the prevention of CMV disease after solid organ transplantation

Organ transplant type	CMV serostatus	General recommendations	Specific recommendations
Kidney, liver, pancreas and heart transplants	D+/R−	Universal prophylaxis is preferred over preemptive therapy Duration of antiviral prophylaxis is 6 months for kidney recipients, and 3–6 months for liver, pancreas and heart recipients Prophylaxis may be prolonged for patients who receive lymphocyte-depleting antibodies for treatment of acute rejection Preemptive therapy may be effective (if performed adequately) but the rapid replication dynamics of CMV in CMV D+/R− patients makes preemptive strategy logistically difficult in this population	Valganciclovir 900-mg QD (not FDA-approved in liver transplantation) is preferred Oral ganciclovir 1-g TID IV ganciclovir 5-mg/kg QD Valacyclovir 8-g/d (kidney transplant recipients only) Some centers add CMV-IG for high-risk patients (in heart transplant patients)
	R+	Either antiviral prophylaxis or preemptive therapy are acceptable options Duration of prophylaxis is 90–100 days Preemptive therapy is guided by PCR or antigenemia testing Other centers do not provide specific anti-CMV prevention strategy and opts to clinically observed low-risk patients (i.e., D−/R+)	*Antiviral prophylaxis*: Valganciclovir 900-mg QD (not FDA-approved in liver transplantation) is preferred Oral ganciclovir 1-g TID IV ganciclovir 5-mg/kg QD Valacyclovir 8-g/d (kidney transplant recipients only) *Preemptive therapy*: Valganciclovir 900-mg BID IV ganciclovir 5-mg/kg q12h
Lung and heart-lung transplants	D+/R−	Universal prophylaxis is preferred over preemptive therapy Duration of prophylaxis is 12 months, and others extend this longer periods	Valganciclovir 900-mg QD IV ganciclovir 5-mg/kg QD Some centers add CMV-IG to the antiviral regimen
	R+	Universal prophylaxis is preferred over preemptive therapy Duration of prophylaxis is 6–12 months	Valganciclovir 900-mg QD IV ganciclovir 5-mg/kg QD
Intestinal transplants	D+/R−	Universal prophylaxis is preferred over preemptive therapy Duration of prophylaxis is 3–6 months; some centers extend the duration beyond 6 months	IV ganciclovir 5-mg/kg QD Valganciclovir 900-mg PO QD Some centers add CMV-Ig to the antiviral regimen
	R+	Universal prophylaxis is preferred over preemptive therapy Duration of prophylaxis is 3–6 months after transplantation	IV ganciclovir 5-mg/kg QD Valganciclovir 900-mg PO QD

CMV cytomegalovirus, *IG* immunoglobulin, *D* donor, *R* recipient, *QD* once daily, *TID* thrice daily, *IV* intravenous.

reduction in the indirect effects of CMV was also demonstrated, such as its impact on other opportunistic infections [177], bacteremia [114], allograft rejection [184–186], and patient survival [184–186, 209]. Table 25-9 lists the current recommendations for the prevention of CMV disease after solid organ transplantation [56, 123, 233, 249].

Ganciclovir and Valganciclovir Prophylaxis

A synthetic analogue of 2′-deoxy-guanosine, ganciclovir exerts its anti-CMV effect through inhibition of CMV DNA polymerase [250, 251]. Ganciclovir requires three sequential phosphorylation steps in order to exert its antiviral activity. The initial step of this triphosphorylation process is carried out by CMV-encoded *UL97* kinase, and two additional phosphate molecules are later added by human cellular kinases [252]. Because of the need for CMV-encoded *UL97* kinase for its initial phosphorylation, ganciclovir becomes activated only in the presence of active CMV infection [252]. Upon its activation, ganciclovir triphosphate acts by competitively inhibiting the incorporation of deoxyguanosine-triphosphate by CMV DNA polymerase. The incorporation of ganciclovir triphosphate by CMV DNA polymerase alters the DNA conformation, which results in the termination of CMV DNA elongation.

The clinical efficacy of ganciclovir for preventing CMV disease after solid organ transplantation is demonstrated by multiple clinical trials that compared it with placebo, acyclovir, or different preparations of immunoglobulins [253–258]. Ganciclovir is available in intravenous and oral formulations [252]. However, oral ganciclovir is poorly absorbed in the gastrointestinal tract. Hence, it is no longer recommended as a first-line agent, and it has been replaced by the highly bioavailable oral valganciclovir [259]. A valyl-ester prodrug of ganciclovir, valganciclovir circumvents the pharmacokinetic limitations of oral ganciclovir and provides systemic ganciclovir levels that are comparable to intravenous ganciclovir [259]. Intraocular injection of ganciclovir is also used as adjunctive treatment of CMV retinitis [101].

Valganciclovir

Valganciclovir is currently the most common antiviral drug for prophylaxis against CMV in solid organ transplant recipients [135]. A prodrug of ganciclovir, valganciclovir is a highly recognized substrate of the intestinal peptide transporter PEPT1, which facilitates its rapid and efficient intestinal absorption [252]. Following absorption, valganciclovir undergoes rapid hydrolysis by intestinal and hepatic esterases into ganciclovir [252]. Because of enhanced absorption, valganciclovir provides serum ganciclovir levels that are comparable to intravenous ganciclovir [259]. The high levels achieved with valganciclovir may reduce the risk of antiviral resistance when compared to oral ganciclovir [260], although ganciclovir-resistance has been reported during prolonged use of valganciclovir.

For antiviral prophylaxis, valganciclovir is administered orally at a dose of 900-mg once daily in individuals with creatinine clearance >60 ml/min (Table 25-9) [135, 252]. In an international randomized controlled trial, valganciclovir (900-mg once daily for 100 days) was noninferior to oral ganciclovir (1-g three times daily for 100 days) for preventing CMV disease in a cohort of 364 CMV D+/R− kidney, pancreas, liver, and heart recipients [135]. The incidence of "endpoint committee-defined CMV disease" were 12.1 and 15.2% at 6 months and 17.2 and 18.4% at 12 months in the valganciclovir and oral ganciclovir groups, respectively [135]. The incidence of "investigator-treated CMV disease" were 30.5 and 28.0% in the valganciclovir and oral ganciclovir groups, respectively [135]. Notably, there was a higher incidence of tissue-invasive CMV disease among liver recipients who received valganciclovir compared to oral ganciclovir prophylaxis [135], and this observation resulted in its non-approval by the US FDA for the prevention of CMV disease after liver transplantation [135]. Despite this, valganciclovir remains as the most common drug used for the prevention of CMV disease after liver transplantation [261]. The incidence of neutropenia was significantly higher among patients who received valganciclovir compared to oral ganciclovir prophylaxis [135]; these findings were correlated with systemic drug levels [262]. Several single-center and retrospective analyses have mirrored these observations by demonstrating the efficacy of valganciclovir prophylaxis after heart, kidney, pancreas, and liver transplantation [28, 29, 133, 159, 263–265]. Clinical trials have also demonstrated the efficacy of valganciclovir for preventing CMV disease after lung transplantation (Table 25-8) [159, 265].

The optimal duration of valganciclovir prophylaxis for the prevention of CMV disease after solid organ transplantation remains debated, although 3 months is considered as the minimum duration for CMV D+/R− kidney, liver, heart, and pancreas recipients [56, 123, 233]. One study of a cohort of 372 CMV D+/R− kidney recipients demonstrated that the incidence of CMV disease is further reduced by doubling the duration from 3 months (100 days) to 6 months (200 days) of valganciclovir prophylaxis (36.8% vs. 16.1%) (Table 25-8) [266]. In another multicenter trial, valganciclovir prophylaxis for 12 months was significantly better than 3 months in reducing CMV disease in CMV D+/R− and CMV R+ lung transplant recipients (Table 25-8) [129, 158, 159]. Table 25-9 lists the recommendations for the prevention of CMV disease in CMV D+/R− and CMV R+ kidney, liver, pancreas, heart, lung, and intestinal recipients [56, 123].

Intravenous Ganciclovir

Clinical trials have demonstrated the efficacy of intravenous ganciclovir for the prevention of CMV disease after solid organ transplantation [255]. However, the need for vascular access and its associated complications of thrombosis, phlebitis, and catheter-related infections have limited the use of intravenous ganciclovir prophylaxis after solid organ transplantation [252]. Oral agents have generally replaced intravenous ganciclovir for prevention of CMV disease. Currently, intravenous ganciclovir remains used as short-course "bridge" prophylaxis during the early period after transplantation, when patients are still unable to take oral medications. The recommended dose for intravenous ganciclovir for prophylaxis is 5 mg per kg per day (dose adjusted based on renal function).

Oral Ganciclovir

Oral ganciclovir was developed to circumvent the limitation of intravenous ganciclovir [132]. For prophylaxis, oral ganciclovir is given at a dose of 1 g orally three times daily for 3 months after solid organ transplantation [56, 132]. The major drawback to oral ganciclovir use is its poor absorption [252, 259]. In a prospective, randomized, placebo-controlled trial involving 304 liver recipients, oral ganciclovir (3 g daily for 98 days) reduced the 6-month incidence of CMV infection from 51.5 to 24.5% and CMV disease from 18.9 to 4.8% [132]. Significant reduction in the incidence of CMV disease was demonstrated even in CMV D+/R− liver transplant recipients (from 44 to 14.8%) and those who received lymphocyte-depleting immunosuppressive drugs (from 32.9 to 4.6%) [132]. Prolonging oral ganciclovir prophylaxis to 6 months further decreased the incidence of CMV disease in kidney recipients [267]. In a single-center observational study, the incidence of CMV disease among patients who received 12 weeks was 31% compared to only 6.5% among patients who received 24 weeks of oral ganciclovir prophylaxis [267].

The prolonged suboptimal systemic levels attained during oral ganciclovir prophylaxis has been postulated as major contributor to the emergence of drug-resistant viral strains, particularly among CMV D+/R− solid organ transplant recipients [260, 268–272]. In an in vitro study, low ganciclovir levels predisposed to the selection of low-grade *UL97* mutations followed thereafter by accumulation of other

mutations [273]. In the clinical setting, persistent CMV replication has been demonstrated despite the use of oral ganciclovir [52]. Although an earlier study showed that ganciclovir prophylaxis in solid organ transplant recipients did not select for ganciclovir-resistant CMV isolates [251], subsequent studies have observed that prolonged use of ganciclovir may predispose to the selection of ganciclovir-resistant CMV [271, 272]. In a study of lung recipients on prolonged ganciclovir therapy, 9% developed ganciclovir resistance at a median of 4.4 months [271]. Currently, oral ganciclovir is no longer a first-line option, but it has been supplanted by valganciclovir for preventing CMV disease after transplantation [261].

25.7.1.2 Acyclovir and Valacyclovir

Acyclovir

Acyclovir was one of the earliest antiviral drugs for the prevention of CMV disease after solid organ transplantation, with modest success (Table 25-8) [244]. In its phosphorylated form, acyclovir acts as a competitive inhibitor of viral DNA polymerase. However, acyclovir possesses little in vitro activity against CMV at clinically achievable levels. In a prospective, randomized, placebo-controlled trial of kidney recipients, oral acyclovir (dose of 800–3200 mg four times daily for 12 weeks) reduced the incidence of CMV infection (36% vs. 61%) and disease (7.5% vs. 29%) compared to placebo [244]. However, other studies have not demonstrated these beneficial effects, particularly in liver and thoracic organ recipients [274, 275]. Thus, it is no longer recommended for the prevention of CMV disease after solid organ transplantation.

Valacyclovir

Valacyclovir, a valyl-ester prodrug of acyclovir, is characterized by improved oral bioavailability [32]. In a prospective, randomized, placebo-controlled trial, valacyclovir (2 g orally four times daily for 90 days) reduced the incidence of CMV disease from 45 to 16% in CMV D+/R− kidney recipients and from 6 to 1% in CMV-seropositive patients (Table 25-8) [32]. Reductions in the incidence of herpes simplex virus infections and allograft rejection were also observed, but hallucinations and mental confusion were prominent adverse effects of high-dose valacyclovir [32]. The clinical utility of valacyclovir prophylaxis has not been demonstrated in recipients of allografts other than kidney [276]. Currently, valacyclovir is indicated only for preventing CMV disease after kidney transplantation (Table 25-9).

25.7.1.3 Foscarnet and Cidofovir

There have been no randomized, placebo-controlled clinical studies of foscarnet and cidofovir prophylaxis for the prevention of CMV disease after solid organ transplantation. Both drugs exert a mechanism of action that is similar to ganciclovir, by inhibiting CMV *UL54 (pol)*-encoded DNA polymerase. Foscarnet is a pyrophosphate analogue that does not require intracellular activation, and terminates CMV replication by blocking the release of pyrophosphate by CMV DNA polymerase. Cidofovir requires a two-step phosphorylation process, catalyzed by human cellular kinases, in order to exert its antiviral effect. In contrast to ganciclovir, both foscarnet and cidofovir do not require phosphorylation by CMV *UL97* kinase. Hence, foscarnet and cidofovir have antiviral activity against ganciclovir-resistant CMV strains with *UL97* mutations [277]. While the long half-life of cidofovir may allow for less frequent and more convenient dosing (e.g., once every 2 weeks), the associated nephrotoxicity and need for parenteral administration are major factors that limited its use. Likewise, foscarnet is administered parenterally and the risk of nephrotoxicity has prevented its use as CMV prophylaxis after solid organ transplantation [277].

25.7.1.4 Delayed-Onset CMV Disease

Antiviral prophylaxis has not completely prevented CMV infection and disease, especially in a subgroup of high-risk CMV D+/R− transplant recipients. Instead, antiviral prophylaxis has merely delayed the onset of disease to a later period, hence the term delayed-onset (late-onset) CMV disease. The onset of delayed-onset CMV disease varies depending on the duration of antiviral prophylaxis. In general, delayed-onset CMV disease occurs mainly during the first 3–6 months after stopping antiviral prophylaxis [28, 29, 32, 56, 132, 133, 135]. The reported incidence of delayed-onset CMV disease varied in different studies, from as low as 8% to as high as 47% of CMV D+/R− solid organ transplant recipients [28, 29, 32, 56, 132, 133, 135, 264].

The clinical presentation of delayed-onset CMV disease appears similar to traditional-onset CMV disease, with the majority of cases presenting as CMV syndrome (estimated >60%) and less commonly as tissue-invasive CMV disease [28, 29, 32, 56, 132, 133, 135, 264]. The most common organ involved is the gastrointestinal tract [28, 29, 32, 56, 132–135, 208, 264]. The severity of illness appears to be comparatively lesser than early-onset CMV disease, possibly as a result of the lower degree of immunosuppression at later posttransplant period. In some cases, the clinical presentation may be atypical and the diagnosis may be missed, leading to death [278]. Delayed-onset CMV disease remains significantly associated with poor allograft and patient survival [29, 134, 208].

The major risk factor for delayed-onset CMV disease is a CMV D+/R− serologic status [28, 29, 32, 56, 132–135, 208, 264]. Viral load monitoring and CMV serology (at the end of antiviral prophylaxis) have not been particularly useful in predicting delayed-onset CMV disease [279, 280]. Routine surveillance using nucleic acid test to monitor early asymptomatic CMV reactivation that can be treated preemptively

before it progresses to clinical disease has been proposed to reduce the incidence of delayed-onset CMV disease, but only with modest efficacy (Figure 25-1c). In contrast, measurement of cell-mediated immunity, such as interferon-gamma release by T cells following CMV antigenic stimulation have been shown to stratify patients that remain at high risk of CMV disease after completion of antiviral prophylaxis [226]; these individuals may benefit from extended antiviral prophylaxis or a more aggressive CMV surveillance post-prophylaxis. The vast majority of delayed-onset CMV disease cases remain susceptible to ganciclovir, although some may be due to drug-resistant strains [281]. Ganciclovir-resistant CMV disease causes significant morbidity and results in poor allograft and patient survival [281–283].

25.7.2 Preemptive Therapy

Preemptive therapy involves the administration of antiviral drugs "selectively" only to patients with asymptomatic CMV reactivation with the main goal of preventing its progression to symptomatic CMV disease [56, 123, 233]. An algorithm for preemptive therapy is depicted in Figure 25-1b, c). Meta-analysis of clinical trials using preemptive therapy for the prevention of CMV disease is listed in Table 25-5. The results of comparative trials between preemptive therapy and antiviral prophylaxis for the prevention of CMV disease after solid organ transplantation are listed in Table 25-6.

For preemptive therapy to work optimally, a sensitive and highly predictive laboratory test should be readily available to identify patients at increased risk of developing CMV disease [56, 136, 257, 284]. Transplant recipients are monitored with this test, usually on a weekly basis, until a "viral threshold" is reached that triggers the initiation of antiviral treatment [136]. For more than a decade, there has been debate as to the optimal method and frequency for CMV surveillance. Among the various laboratory methods used for the diagnosis of CMV (Table 25-4) [136], those that have been shown to be effective for guiding preemptive therapy are pp65 antigenemia [257, 285–288] and nucleic acid tests [238, 284, 289–292]. Which of these laboratory methods is more effective in guiding preemptive therapy is dependent on a variety of factors. The low sensitivity and slow turnaround time of culture-based assays have limited their use for preemptive therapy, and are strongly discouraged for this purpose [293, 294]. The optimal frequency of CMV surveillance by CMV nucleic acid test or pp65 antigenemia has been suggested to be once weekly for the first 12 weeks after solid organ transplantation [56, 123, 233, 249].

The viral load threshold for initiating preemptive antiviral therapy has not been well defined. Differences in patient characteristics, lack of standardization among laboratory assays, and the varying analytical performance of diagnostic tests have made it difficult, if not impossible, to define a specific clinically significant level of viral replication [136, 213, 295]. Recent studies using standardized nucleic tests have suggested a viral load ranging between 2000 and 5000 IU/ml as threshold for initiating preemptive antiviral therapy for CMV R+ solid organ transplant recipients, while any viral load value may be significant for the CMV D+/R− solid organ transplant recipient [218]. These suggested values should be confirmed by others before it becomes widely adopted. Likewise, a clinically relevant cutoff value of pp65 antigenemia has not been widely defined nor adopted. A suggested threshold of >10 positive cells per 2×10^5 cells in solid organ transplant recipients has yet to be validated by prospective clinical trials [296]. Pending widespread confirmation of these suggested viral thresholds, it is imperative for individual transplant centers to develop clinically validated thresholds that will trigger the initiation of preemptive therapy. It is likely that the suggested threshold may differ between CMV D+/R− and R+ patients, between lung and non-lung transplant recipients, and between those who received or did not receive induction therapy with lymphocyte-depleting agents, among other factors.

Intravenous ganciclovir and oral valganciclovir are most commonly used antiviral drugs for preemptive antiviral therapy (Table 25-9) [297]. In a randomized trial, intravenous ganciclovir and oral valganciclovir were equally effective for the preemptive treatment of CMV infection [297]. High-dose oral acyclovir, intravenous acyclovir, valacyclovir, and immunoglobulin preparations should not be used for preemptive therapy since they are comparatively less effective than ganciclovir-based regimens in treating active CMV replication [56, 123, 233, 249]. Oral ganciclovir has been used successfully for preemptive therapy in some clinical studies [256, 284, 292, 298], but its poor absorption, the risk of emergence of ganciclovir-resistant CMV in the presence of low systemic ganciclovir levels, and the availability of more bioavailable valganciclovir has limited (and should discourage) the use of preemptive oral ganciclovir therapy. Because of associated severe toxicities, foscarnet and cidofovir are not used as first-line agents for preemptive treatment in solid organ transplant recipients [256, 258, 284].

The duration of antiviral administration with preemptive therapy is guided by CMV surveillance using nucleic acid test or antigenemia testing [56, 123, 233, 249]. It is generally recommended to continue preemptive antiviral treatment until CMV is no longer detectable in the blood for at least 2 weeks [56, 123, 233, 249]. CMV surveillance is recommended on a weekly basis for the duration of preemptive treatment and for the entire "high-risk" period (i.e., 12 weeks after transplantation) [56]. CMV testing would provide an indication of the efficacy of the antiviral treatment, with a decline in viral load as an indication of effective treatment, while non-decline or rising viral load is an indication of antiviral drug resistance or poor drug bioavailability [271, 272, 281, 299]. It is important to recognize, however, that viral load may occasionally transiently rise during the first 1–2

weeks of preemptive treatment, and this does not necessarily suggest antiviral drug resistance [300].

Table 25-9 lists the recommendation for using preemptive therapy for the prevention of CMV disease after solid organ transplantation. The rapid CMV replication in CMV D+/R− solid organ transplant patients has led many centers to question the implementation of preemptive therapy in this high-risk population [36, 37, 106]. In few studies [284, 292, 298], many CMV D+/R− solid organ transplant patients were not identified soon enough for the timely initiation of preemptive treatment. Hence, current guidelines have preferred antiviral prophylaxis over preemptive therapy in CMV D+/R− solid organ transplant recipients [56, 123, 233, 249]. Other centers however have reported good experience with preemptive therapy even in this high-risk CMV D+/R− population [256, 257, 301].

The benefits and disadvantages of preemptive therapy are listed in Table 25-7. Compared to antiviral prophylaxis, wherein antiviral drugs are administered to all at-risk patients, preemptive therapy will provide antiviral drugs only selectively to patients with asymptomatic CMV infection [302]. Accordingly, the duration of antiviral drug administration is relatively shorter and fewer patients will receive the antiviral drug; this has the potential advantage in reducing drug costs and adverse effects. Moreover, preemptive therapy may be associated with a lower risk for emergence of resistant strains, although ganciclovir-resistant CMV has been observed among transplant recipients who received prolonged preemptive therapy [271, 303].

25.7.3 Targeted Prophylaxis

Targeted prophylaxis involves the administration of antiviral drugs to selected patients with clinical and epidemiologic characteristics that heightened their risk of CMV disease [56, 80, 108, 304–307]. The principle of this prevention approach is that the intensity of antiviral strategy should parallel the intensity of the antirejection program. The most important risk is the use of lymphocyte-depleting anti-T-cell receptor antibodies, either as induction therapy or for treatment of allograft rejection [56, 80, 108, 307]. Intravenous ganciclovir therapy given during anti-lymphocyte antibody therapy has been shown to decrease the incidence of CMV disease from 33 to 14% in CMV R+ kidney transplant recipients [307, 308]. Currently, valganciclovir is the most commonly used drug for targeted prophylaxis. Intravenous and oral ganciclovir are alternative agents. In contrast, immunoglobulins, acyclovir, and valacyclovir are not generally recommended for targeted prophylaxis. The optimal duration of targeted prophylaxis is not well defined, although many centers provide antiviral therapy for 1–3 months following the use of anti-lymphocyte antibody therapy [56, 123, 233, 249].

25.7.4 Other CMV Prevention Approaches

25.7.4.1 Cytomegalovirus-Seronegative Blood Products and Protective Matching

The CMV serologic status of the donor and recipient prior to solid organ transplantation is an important predictor of the risk of CMV disease, with CMV D+/R− representing the highest risk [309–311]. The use of protective matching (i.e., transplantation of an allograft from a seronegative donor to a seronegative recipient; D−/R−) markedly reduces the risk of CMV disease in the seronegative recipient by avoiding the transmission of CMV during solid organ transplantation [309–311]. The overall rate of CMV disease during the first year after solid organ transplantation in CMV D−/R− is up to 1–2% [121]. However, this "selective" approach is impractical, and rarely used. Waiting for a CMV-seronegative donor is likely to be risky and detrimental since it may delay a life-saving transplant procedure. Moreover, the approach does not guarantee complete protection since natural transmission of CMV occurs in the community.

CMV-seronegative or leuko-reduced blood products are suggested for transplant recipients, especially CMV D−/R− transplant recipients, who require blood transfusions for any indication [121, 309–312]. Because majority of blood donors are CMV-seropositive, one limitation is the scarcity of CMV-seronegative blood products. In such a situation, filtered or leukocyte-poor products, which are associated with a low risk of CMV transmission, have been recommended [312]. In studies that compared CMV-negative vs. leuko-reduced blood products, there was no significant difference in the incidence of transfusion-transmitted CMV infection [310, 313–315].

25.7.4.2 Immunotherapy and Vaccination

In theory, one intervention to reduce the risk of CMV infection after solid organ transplantation is CMV vaccination [316–319]. In the 1980s, randomized, placebo-controlled, double-blind trials of the live-attenuated Towne vaccine in kidney recipients demonstrated safety and immunogenicity, but did not demonstrate significant prevention of CMV infection, although reduction in CMV disease severity by up to 85% was observed [320–322]. Moreover, among CMV D+/R− subgroup, the 1- and 5-year graft survival rates were higher in CMV-vaccinated patients (73 and 62%, respectively) compared to placebo (40 and 25%, respectively) [323]. Currently, there are major efforts to develop an effective CMV vaccine [324–327], including peptide-based, DNA-based, and subunit vaccines containing recombinant immunodominant glycoproteins [316–319, 328]. The glycoprotein B vaccine with MF adjuvant has reduced CMV infection in healthy women [329], while the ASP0113 vaccine is undergoing phase II–III clinical trials in transplant

recipients [330]. Currently, no vaccine is approved for clinical use.

The administration of unselected or CMV immunoglobulins have been used to boost humoral immune response in an attempt to prevent CMV disease after solid organ transplantation [159, 331–337]. Results from randomized, controlled trials in kidney recipients indicate that immunoglobulins prevented CMV disease in some, but not all trials [159, 331–337]. Two meta-analyses demonstrated that immunoglobulin prophylaxis was associated with reduction in CMV-related death [338, 339]. However, one study showed no significant reduction in CMV disease, CMV infection, and all-cause mortality when compared to placebo or no treatment [339]. This study further suggested that immunoglobulins did not have additional protective benefit to that provided by antiviral drugs alone [339]. A more recent analysis of a large database confirms the observation that while CMV-immunoglobulins with or without antivirals was associated with reduced risk of death or graft loss, this similar finding was observed with the use of antivirals alone [340]. Despite these data, some centers use unselected or CMV-immunoglobulins as "adjunctive" agent for the prevention of CMV disease in high-risk populations such as lung, heart, and intestinal transplants, in combination with antiviral drugs [56, 123, 233, 249]. The high cost of immunoglobulins and the risks associated with parenteral administration are the major disadvantages of immunoglobulins.

25.8 Treatment of Cytomegalovirus Disease

CMV disease in solid organ transplant recipients is a potentially fatal illness that should be treated aggressively as soon as possible. The antiviral drugs for treating CMV disease are intravenous ganciclovir, oral valganciclovir, intravenous foscarnet and intravenous cidofovir. All these drugs inhibit CMV by interference of the function of *UL54 (pol)*-encoded CMV DNA polymerase. Intravenous ganciclovir and valganciclovir are considered the first line agents and are similarly effective for the treatment of mild to moderate CMV disease in solid organ transplant recipients [138]. Because of significant toxicities, foscarnet and cidofovir are considered second line alternative drugs (Table 25-10) [56]. Foscarnet and cidofovir are reserved for the treatment of ganciclovir-resistant CMV disease. In addition to antiviral therapy, treatment of CMV disease should be complemented by a judicious reduction in pharmacologic immunosuppression.

25.8.1 Intravenous Ganciclovir and Valganciclovir

Clinical trials have demonstrated the efficacy and safety of intravenous ganciclovir for the treatment of CMV disease after solid organ transplantation [138, 233, 341–343]. The administration of intravenous ganciclovir resulted in a significant decline in the CMV DNA levels, which accompanied clinical resolution of symptoms [137, 138, 279, 295, 341, 344]. The half-life of CMV, which is a measure of the rate of CMV decline, during intravenous ganciclovir therapy ranges from 2.36 days in liver recipients [106] to as long as 5 days in a heterogenous group of solid organ transplant patients [279, 344]. The standard therapeutic dose of ganciclovir is 5 mg per kg every 12 h. Because ganciclovir is excreted by the renal system, patients with renal impairment require dose reductions [345].

The oral formulation of ganciclovir should not be used for treatment of CMV disease because its oral bioavailability is very low and the level achieved in the blood is not sufficient to treat active CMV replication [252, 259]. In contrast, oral valganciclovir is characterized by significantly improved bioavailability with serum ganciclovir concentrations that are ten times higher than oral ganciclovir and approximate those achieved with intravenous ganciclovir

Table 25-10. Antiviral drugs for the treatment of CMV disease after solid organ transplantation

Drug	Dose and duration[a]	Mechanism of action	Common adverse events	Comments
Ganciclovir	5 mg/kg IV every 12 h	Inhibits CMV DNA polymerase	Bone marrow suppression	First-line drug Oral ganciclovir is not readily absorbed and should not be used for treatment
Valganciclovir	900-mg PO twice daily	Inhibits CMV DNA polymerase	Bone marrow suppression	First-line drug for mild-to-moderate cases of CMV disease
Foscarnet	60 mg/kg IV every 8 h (or 90-mg/kg every 12 h)	Inhibits CMV DNA polymerase	Electrolyte abnormalities Nephrotoxicity Anemia Seizure	Second line antiviral drug; utilized in ganciclovir-resistant CMV cases
Cidofovir	5 mg/kg IV every week × 2 weeks then every 2 weeks thereafter	Inhibits CMV DNA polymerase	Nephrotoxicity Ocular hypotony Neutropenia	Rarely used as initial therapy; utilized in ganciclovir-resistant cases

CMV cytomegalovirus.

[a]All dosing should be adjusted based on renal function. The duration of therapy must be tailored as a function of the degree of viral replication, as assessed by CMV DNA PCR or pp65 antigenemia assay, and clinical response (i.e., resolution of symptoms).

[252, 259]. A prospective, randomized, multicenter trial showed that valganciclovir was as effective as intravenous ganciclovir for the treatment of non-severe cases of CMV disease in solid organ transplant recipients [138]. The rates of virological decline and clinical resolution by end of a predefined 21-day treatment course and by the end of maintenance valganciclovir treatment were similar between valganciclovir and intravenous ganciclovir [138]. The recommended dose of valganciclovir for treatment of CMV disease is 900-mg twice daily, with dose adjustments based on renal function. Valganciclovir is not indicated as first line therapy in severe cases of CMV disease, those with very high initial viral load, and in patients whose intestinal absorption is uncertain, such as those with vomiting and diarrhea [252]. In these cases, it is recommended to initiate therapy with intravenous ganciclovir followed by, when the clinical situation improves, step-down therapy with treatment doses of oral valganciclovir.

The duration of antiviral therapy should be individualized based on clinical and virologic response to treatment. The minimum duration of treatment is 2 weeks [56], but most patients require prolonged duration of therapy, especially those with severe disease, tissue-invasive disease, high viral load, and augmented immunosuppression. CMV nucleic acid test or pp65 antigenemia are generally performed once weekly during treatment to assess virologic response to antiviral therapy [56, 123, 233, 249]. CMV monitoring should be performed using the same assay, since viral load values can differ among various tests, especially if they have not been calibrated to the international standard [123]. Studies on CMV kinetics indicate that the degree of viral replication, as measured by the virus load in the blood at the start and end of antiviral therapy, and the degree of viral decay, influence the duration of therapy duration [104, 279, 341]. In a study of 267 solid organ transplant recipients, those patients with viral load higher than 18,200 IU/ml had significantly longer period to CMV disease resolution compared to those with lower viral load [137]. Clearance of viremia (i.e., undetectable viral load) is a useful guide for the discontinuation of antiviral therapy [137, 279, 341]. A study of liver recipients demonstrated that a detectable virus load at the end of antiviral therapy predicted the occurrence of CMV relapse [341]. In contrast, viral load decline to <137 IU/ml was significantly associated with clinical CMV disease resolution [137]. Clinical experience suggests that longer durations of treatment are required in patients with end-organ CMV disease, such as pneumonitis, retinitis, and gastrointestinal CMV disease [56]. Such prolongation of antiviral treatment with end-organ disease is due to the longer persistence of the virus in the tissues compared to the blood [146]. In a study of gastrointestinal disease, CMV remains detectable in tissue for at least 1 week after it has been cleared from the blood [146]. One important limitation of viral load as a clinically useful indicator of the duration of antiviral therapy is in cases of "compartmentalized" organ-invasive diseases, which are characterized by minimal or transient viremia (e.g., retinitis, some cases of hepatitis and gastrointestinal diseases) [27, 101]. Compartmentalized CMV disease is generally more common in CMV R+ solid organ transplant recipients with late onset gastrointestinal CMV disease [225].

The efficacy of maintenance antiviral therapy, wherein antiviral drugs are given at prophylactic doses following a full-dose induction treatment, is debated. Some authorities recommend their use in order to reduce the risk of CMV relapse. However, clinical data indicate that the rate of CMV disease relapse did not differ significantly between the group who received or did not receive secondary maintenance prophylaxis [146, 346]. Some experts have continued to recommend the use maintenance therapy in clinical situations where the degree of immunosuppression is high [121].

Recurrence of CMV infection and disease occurs in up to 25–35% of solid organ transplant recipients with CMV disease [341, 343]. CMV recurrence has been significantly correlated with incomplete clearance of virus from the blood at the end of treatment (i.e., the duration of treatment may have been insufficient). In addition, the immunologic condition of the host may influence the risk of relapse, and patients with persistent severe immunocompromise (i.e., CMV-specific T cell deficiency) are more likely to have recurrence of CMV infection and disease after cessation of antiviral therapy. Most cases of recurrent CMV disease respond to retreatment with intravenous ganciclovir [341, 343]; however, a few cases may be due to ganciclovir-resistant CMV.

The adverse effects of intravenous ganciclovir and valganciclovir include leukopenia, neutropenia, thrombocytopenia, anemia, eosinophilia, bone marrow hypoplasia, hemolysis, nausea, diarrhea, renal toxicity, seizures, mental status changes, fever, rash, and abnormal liver function tests [56, 135, 138, 252]. Thus, hematologic profile and liver and renal function should be monitored once weekly while the patient is receiving ganciclovir. Ganciclovir has teratogenic and carcinogenic potential, and gonadal toxicity has been shown in animal models [56, 135, 138, 252].

25.8.2 Foscarnet

Compared with intravenous ganciclovir and oral valganciclovir, there is limited clinical data on the use of foscarnet for the primary treatment of CMV disease in solid organ transplant recipients [233, 277, 347]. Because of the high risk of toxicity, foscarnet should be reserved for patients intolerant of ganciclovir (such as in cases of severe leukopenia not responsive to granulocyte colony stimulating factors) or for those who failed ganciclovir therapy as a result of drug-resistance. Because foscarnet does not require *UL97* phosphotransferase-mediated chemical modification for antiviral activity (see Antiviral Resistance below), it is the drug of choice for the treatment of *UL97*-mutant ganciclovir-resistant CMV disease [233, 277, 347]. Foscarnet is administered intravenously at a

dose of 60 mg per kg every 8 h (or 90 mg/kg twice daily); patients with renal insufficiency require dose adjustment. The major adverse effects of foscarnet are nephrotoxicity (e.g., acute tubular necrosis, interstitial nephritis, or the precipitation of crystals in the glomerular capillaries), hemorrhagic cystitis, urethral ulcerations, anemia, hyperphosphatemia, hypophosphatemia, hypercalcemia, hypocalcemia, hypomagnesemia, nausea, vomiting, and seizures [348]. The high incidence of electrolyte disturbances warrants aggressive monitoring and repletion, as needed.

25.8.3 Ganciclovir-Foscarnet Combinations

Small studies have investigated combined therapy with ganciclovir and foscarnet on the basis of in vitro data that suggest these agents have synergistic antiviral activity [349]. An small observational study on solid organ transplant recipients suggest that combined ganciclovir and foscarnet, with each administered at half doses, is effective in treating ganciclovir-resistant CMV infection [350]. However, a larger trial that evaluated a mixed group of bone marrow and solid organ transplant recipients demonstrated that the adverse events were more commonly observed among those patients receiving combination therapy, even at reduced doses, than among those receiving a single drug [351]. Moreover, when half doses are used, the risks associated with lower serum drug concentrations, such as fostering drug resistance, must be considered. Current guidelines do not recommend their use for the treatment of CMV disease [121].

25.8.4 Cidofovir

Cidofovir, a phosphonomethoxy analogue of cytosine, is used much less commonly than ganciclovir and foscarnet for the treatment of CMV disease in solid organ transplant recipients [121, 277]. Its long half-life offers the benefit of convenient once weekly dosing frequency. However, its nephrotoxicity has limited its clinical use. As with ganciclovir and foscarnet, it acts by inhibiting *UL54 (pol)*-encoded CMV DNA polymerase [277]. Like foscarnet, cidofovir does not require *UL97*-mediated phosphorylation for its activation and could potentially be used for the treatment of *UL97*-associated ganciclovir resistant CMV disease [277]. Mutations in DNA polymerase (*UL54*), however, would potentially confer cross-resistance among the three anti-CMV drugs [277].

25.8.5 Immunoglobulin Preparations

CMV hyperimmunoglobulin was used for the treatment of CMV infection before the availability of ganciclovir and other effective antiviral agents [332, 337, 339, 352]. However, data on its efficacy for the treatment of CMV disease, when effective antiviral drugs are standard of care, is conflicting. While some investigators found it ineffective in the treatment of CMV disease [353], others report potential efficacy as adjunct to antiviral therapy in patients with severe CMV disease [121, 334, 354]. One study reported that the combination of CMV immunoglobulin with intravenous ganciclovir was efficacious in the treatment of CMV disease [355]. However, the lack of large randomized, controlled studies addressing its efficacy, the expense of immunoglobulin, and the current availability of effective and less expensive antiviral drug alternatives have limited its widespread clinical use. Nonetheless, combinations of antiviral drugs and immunoglobulin therapy may be useful in certain subsets of patients, particularly in transplant recipients presenting with severe CMV diseases such as CMV pneumonitis [56, 123, 233, 249].

25.8.6 Adoptive CMV-Specific T-Cell Therapy

Because CMV-specific cell mediated immunity is required to control CMV disease, a strategy to boost CMV-specific immunity after solid organ transplantation may have a beneficial effect in decreasing CMV disease. Studies have correlated CMV disease with a lack or diminished CMV-specific T cells [321]. Thus, expanding the number and function of virus-specific cytotoxic T-lymphocytes could reduce the incidence of CMV disease, although this approach remains investigational. There is more clinical experience of this approach in allogeneic hematopoietic stem cell transplant recipients, but adoptive transfer of CMV-immune T cells have been used in a series of solid organ transplant recipients with multidrug-resistant CMV infection [356, 357].

25.8.7 Novel and Off-Label Therapeutics

Several novel drugs are currently being tested for their efficacy against CMV in solid organ transplant recipients. A lipid formulation of cidofovir, brincidofovir (CMX001), acts by inhibiting *UL54 (pol)*-encoded CMV DNA polymerase. In contrast to the nephrotoxic intravenous cidofovir, brincidofovir is given orally and does not exhibit renal toxicity [358]. It is currently undergoing clinical trials in transplant recipients with early phase studies in hematopoietic stem cell transplant recipients [358].

Letermovir is a novel drug that is currently in phase III clinical trials for the management of CMV disease in solid organ transplant recipients [359, 360]. It acts by inhibiting the viral terminase complex, which occurs at a step later than DNA synthesis, and prevents the incorporation of the CMV DNA molecules into viral capsids. Letermovir has been tested in a phase IIa clinical trial in a small cohort of kidney recipients, where it was found to be safe and with potential efficacy that is comparable to the standard preemptive treat-

ment of CMV infection [359]. Its main toxicities are nausea, vomiting, and diarrhea [359, 360].

Maribavir is a benzimidazole drug that inhibits *UL97* of CMV. Results of early clinical trials have been inconsistent. While the results of a phase II clinical trial in hematopoietic stem cell transplant recipients showed safety and potential efficacy [361], a phase III trial demonstrated it is less active than oral ganciclovir for preventing CMV disease in CMV D+/R− liver recipients [362]. However, maribavir has been used anecdotally, with some success, for the treatment of a series of multidrug-resistant CMV disease cases in solid organ transplant recipients [363, 364]. After a temporary halt in its clinical development as a result of the disappointing phase III trials, maribavir has resumed its clinical evaluation for the treatment of CMV disease in solid organ transplant recipients.

The antimalarial drug, artesunate, has been used off-label for the treatment of CMV disease in transplant recipients, but with inconsistent results [365, 366]. Leflunomide, a pyrimidine synthesis inhibitor, has also been used to treat a series of cases with multidrug-resistant CMV infection, but also with inconsistent results [367].

25.9 Antiviral Resistance

Drug resistance has emerged as an important clinical problem in solid organ transplant recipients with CMV disease. Drug resistance has been observed against all approved antiviral agents (ganciclovir, cidofovir and foscarnet) but the most common form involves resistance only to ganciclovir.

The major mechanisms underlying phenotypic resistance to ganciclovir are specific mutations in *UL97* kinase and less commonly, *UL54 (pol)* DNA polymerase [273, 368, 369]. Mutations in *UL97* kinase that confer ganciclovir resistance involve amino acid substitutions or deletions in specific regions of *UL97* (codons 460, 520, 590–607), and are associated with varying levels of phenotypic resistance [273]. The seven most commonly reported *UL97* mutations are M460I, M460V, H520Q, C592G, A594V, L595S, and C603W [370]. *UL97* encodes for a viral kinase that catalyzes the first step in the triphosphorylation of ganciclovir into the active ganciclovir triphosphate. The net effect of *UL97* mutations is an absent or low level of triphosphorylated (active) ganciclovir within CMV infected cells, and a resultant decreased inhibition of viral DNA polymerase. Depending on the type of *UL97* mutation, it may result in low-level or high-level resistance to ganciclovir [370]. Since *UL97* kinase is not involved in the pharmacodyamics of foscarnet and cidofovir, *UL97* mutations do not confer cross-resistance to cidofovir or foscarnet. Much less commonly, antiviral drug resistance may be conferred by mutations in *UL54 (pol),* which encode for CMV DNA polymerase. Isolated *UL54 (pol)* mutations are observed rarely, and they occur more commonly as second step mutations, after mutations in *UL97* have already occurred. Because *UL54 (pol)* encodes for the polymerase target of all three drugs, there is potential cross-resistance among ganciclovir, foscarnet and cidofovir in patients infected with *UL54*-mutant CMV strain [271, 272, 281, 283, 368, 370–374]. The degree of cross-resistance will vary depending on the *UL54* codon segment that is affected [370]. Cross-resistance is more often observed between ganciclovir and cidofovir, compared to ganciclovir and foscarnet [370]; hence, foscarnet is the first-line agent when ganciclovir resistance is suspected [121].

The single most important variable associated with drug-resistant CMV is a CMV D+/R− mismatch status. The vast majority of solid organ transplant recipients with ganciclovir-resistant CMV infection have been CMV seronegative prior to transplant [271, 272, 281, 373–375]. The estimate, among CMV D+/R− patients, of ganciclovir resistance in various studies has been 5–10%. Other factors associated with ganciclovir resistance are high viral load, augmented immunosuppression, and prolonged low-level antiviral drug exposure [374]. The incidence is also higher in lung and pancreas compared to other organ transplants, potentially reflecting the severity of drug-induced immune suppression [374].

There is an apparent fitness disadvantage of drug-resistant mutant CMV [105, 106], but these strains do not loss their replication ability and they remain fully pathogenic based on reports of progression to clinical disease despite full-dose ganciclovir therapy, demonstration of resistant strains directly in diseased tissue, and an association of resistant strains with the full spectrum of clinical CMV disease seen with wild-type susceptible strains [272, 376]. In addition, there is an association between drug-resistant CMV and higher morbidity [281, 375] and decreased survival [281, 282, 375, 377], when compared to wild-type susceptible CMV strains.

Antiviral resistance should be suspected in transplant recipients with risk factors (as discussed above) and an inadequate virologic and/or clinical response after 2 weeks of full-dose antiviral therapy. Historically, the diagnosis of antiviral resistance had been confirmed by demonstration of decreased susceptibility of clinical viral isolates by plaque reduction or other methodologies. However, these assays are cumbersome and have slow turnaround time, lack standardization, and thus have limited clinical availability. Currently, genotypic assays for *UL54* and *UL97* mutations directly from clinical specimens are the diagnostic tests of choice when drug-resistant virus is suspected in the clinical setting [121, 378, 379].

Treatment of drug-resistant CMV is based largely on expert opinion, and data to support the practice is supported mainly by anecdotal experiences. The choice of empiric antiviral therapy should account for theoretical considerations such as prior antiviral drug exposure, severity of disease, concurrent medical conditions (renal function), the organ transplanted, and degree of immunosuppression. In general, these patients should undergo reduction in pharmacologic

immunosuppression, if feasible. Depending on the disease severity and other risk factors, the initial empiric regimens have mostly included a switch to foscarnet therapy for those with severe symptoms [208, 281, 380]. Foscarnet is the preferred empiric choice for treatment of ganciclovir-resistant CMV strains, since *UL54 (pol)* mutation commonly confers cross-resistance between ganciclovir and cidofovir [370]. Mild clinical disease may be empirically treated with higher than standard regimens of intravenous ganciclovir (as high as 7.5–10 mg/kg every 12 h) [381]. Other treatment interventions have included adjunctive intravenous immunoglobulin or CMV-hyperimmunoglobulin [272, 281], leflunomide [380, 382–384], artesunate [367], and switch to sirolimus immunosuppression [385]. Alternative antiviral agents in various stages of clinical development that have shown in vitro activity against ganciclovir-resistant CMV strains are brincidofovir, letermovir, and maribavir. Definitive antiviral therapy can be chosen as directed by *UL97* and *UL54* genetic testing. As with the treatment of many transplant-related opportunistic infections, it is highly recommended that a reduction in pharmacologic immunosuppression be undertaken to complement the management of drug-resistant CMV disease.

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26
Epstein–Barr Virus Infection and Lymphoproliferative Disorders After Transplantation

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Epstein–Barr virus (EBV) is among the most successful of human pathogens, infecting virtually the entire human population and persisting throughout the lifetime of its host. Having coevolved with man over millions of years, infection with EBV is most often benign despite the fact that EBV is one of the most potent transforming viruses in vitro and is the prototypical human tumor virus. EBV is strongly associated with the development of a wide variety of neoplasms, including lymphoid tumors such as Burkitt lymphoma, Hodgkin lymphoma, T and/or natural killer (NK) cell lymphoma, and immunosuppression-related lymphoproliferative disorders, epithelial malignancies such as undifferentiated nasopharyngeal carcinoma and a distinct subset of gastric carcinomas, and a smooth muscle tumor, leiomyosarcoma [1, 2].

EBV's ability to persist in the host, establish latency, and transmit infection arises from a finely balanced equilibrium between the virus and the host's immune response, particularly the EBV-specific cytotoxic T lymphocyte (CTL) response as reviewed by Taylor et al. [3]. In recipients of hematopoietic stem cell transplants (HSCTs) and solid organ transplants (SOTs), the use of increasingly potent and T cell-targeted immunosuppression disrupts this balance in favor of the virus. The proliferation of EBV-infected lymphoid cells that may result leads to a highly diverse spectrum of disease known as posttransplant lymphoproliferative disorders (PTLD) [4, 5]. PTLD has emerged as an increasingly important complication that affects both graft and patient survival.

In this chapter, the term PTLD is used to describe all clinical syndromes arising from lymphoproliferation, both driven by EBV and of unknown etiology, ranging from a benign self-limited form of polyclonal proliferation to true malignancies containing clonal chromosomal abnormalities, consistent with the World Health Organization (WHO) lymphoma classification system [6]. PTLD requires pathology for definitive diagnosis, but EBV can cause symptoms and signs in SOT and HSCT recipients in the absence of mass lesions. These syndromes are often designated as EBV "disease" when laboratory evidence for EBV infection exists and other causes have been eliminated. This chapter reviews the pathogenesis of EBV-associated disease and PTLD, the risk factors for PTLD development, and strategies that may permit early diagnosis, management, and prevention of this complication.

26.1 The Biology of Epstein–Barr Virus Infection

EBV, a herpesvirus of the γ (gamma)-1 or lymphocryptovirus genus, is an enveloped icosahedral virus with a 170–180 kb linear double-stranded DNA and a primate-restricted host range [1]. Transmission of EBV infection occurs primarily by saliva exchange. In the lower socioeconomic strata and developing nations, EBV infection is almost universally acquired in early childhood and is usually asymptomatic. Infants appear to be relatively protected from infection very early in life; passive maternal antibody may play a role [7]. In industrialized nations, particularly among the upper socioeconomic strata, subjects are often infected in adolescence and early adult life. A recent prospective study of EBV-naïve college students experiencing primary EBV found that only 11% were asymptomatic, with 77% experiencing the infectious mononucleosis syndrome [8]. Infectious mononucleosis is believed to be an immunopathological disease where symptoms are the result of an exaggerated T-cell response to a self-limited lymphoproliferative process. Although maturation of the immune system or heterologous immunity may contribute to the increased T-cell activation, other risk factors for symptomatic disease may include the initial viral inoculum, viral type (type 1 vs. type 2), and polymorphisms of HLA class 1 and immune response genes [3, 9, 10]. Even in Western industrialized societies, more than 90% of the population has immunity to EBV by the age of 40.

The naïve B cell is the primary target of EBV and the memory B cell is the site of EBV persistence. Host–pathogen détente is achieved with ongoing low-grade replication

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TABLE 26-1. Epstein–Barr virus transcription programs found in vivo

Latency	Genes expressed	Proposed function	Expression site normal host	Associated malignant conditions
Growth program (latency III)	*mRNA* EBNA-1,2,3s, LP LMP-1 LMP-2A, 2B *Noncoding RNA* EBERs BART miRNA BHRF1 miRNA	Activates a resting B cell to become a proliferating lymphoblast	Naïve B cell of tonsil	Immunodeficiency (congenital, HIV, PTLD[a])-related lymphoproliferative disorders
Default program (latency II)	*mRNA* EBNA-1 LMP-1 LMP-2A *Noncoding RNA* EBERs BART miRNA	Provides necessary survival signals for: 1. Infected lymphoblasts to differentiate into memory, and 2. Maintenance of persistently infected memory cells	Germinal center and memory cells of the tonsil	Hodgkin lymphoma, nasopharyngeal carcinoma, T-cell lymphoma, PEL
(Latency 1)	*mRNA* EBNA-1 *Noncoding RNA* EBERs BART miRNA	Ensures replication of viral genome during cell division	Dividing memory cells in peripheral blood	Burkitt lymphoma
Latency program (latency 0)	*Noncoding RNA* EBERs BART miRNA	Allows virus persistence in resting recirculating memory cells in nonpathogenic form, not detectable by the immune system	Memory cells in peripheral blood	
Lytic program	>80 Genes expressed	Viral replication Immune evasion by expression of proteins that modulate immune cell function, antigen presentation or apoptotic pathways	Plasma cells of the tonsil, oropharyngeal epithelial cells	Expressed within a minority of cells within most EBV-positive PTLD (diffuse large B cell lymphoma)

EBNA Epstein–Barr virus nuclear antigen, *EBER* EBV-encoded RNA, *BARTs* Bam H1 rightwards transcripts, *LMP* latent membrane proteins, *HIV* human immunodeficiency virus, *PTLD* posttransplant lymphoproliferative disorder, *PEL* primary effusion lymphoma.
[a]At an individual cell level in PTLD, latency patterns may be more variable with some cells expressing latency I, II, or a pattern that includes expression of EBNA-2 but not LMP-1 (designated as latency II-b).

in the oropharynx occurring simultaneously with a latent infection of B cells in the peripheral blood and lymphoid tissues despite the presence of strong humoral and cell-mediated immune responses to the virus. Both a "direct infection" model [1, 11] and a "germinal center" model have been used to explain persistent EBV infection [12, 13]. Controversial and unresolved issues in the theoretical models exist and have been discussed elsewhere [1, 11, 13]. In the germinal center model explained here, EBV usurps normal physiologic B-cell responses by expressing different gene "programs" depending on the location and differentiation state of the B cell (Table 26-1).

Infection of susceptible naïve B lymphocytes occurs primarily within the follicular mantle of the tonsil after the virus replicates in or is transcytosed across epithelial cells. B-cell infection requires binding of the major EBV outer envelope glycoprotein gp350/220 with the cellular complement receptor C3d (also known as CD21 or CR2) and gp42 with the major histocompatibility complex (MHC) class II molecule. Cell fusion is mediated by gH, gL, and gB (reviewed by Ref. [14]). The viral DNA is transduced into the cell in a naked, chromatin-free state and in the initial prelatent phase, an abortive lytic infection occurs that is believed to play a role in initial B-cell activation, cell cycle entry and immune evasion (reviewed in Ref. [15]). The viral DNA is circularized (episome) and methylated. This is followed by expression of a limited subset of latent viral genes as the "growth program" (latency III) as reviewed in Refs. [1, 12] (Table 26-1). This efficiently drives the B cells to become activated proliferating lymphoblasts by mimicking the signals of T-helper (TH) cells, antigen-presenting cells, and regulatory cytokines usually required for B-cell activation.

Proliferating blasts are not ordinarily a pathogenic threat because these cells migrate to the follicle, go through a germinal-center reaction, and then differentiate out of the cell cycle into a resting state by becoming a memory B cell [12, 13]. This requires a switch to the "default program" (latency II) (Table 26-1). Thus, the latently infected B cell

survives activation to enter the peripheral blood as a $CD27^+$ $sIgD^-$ memory B cell (mB_{Lat}), phenotypically resembling a cell that has undergone antigen selection. It contains one to two genome copies per nucleus and contains EBERs and BART miRNAs but expresses no EBV proteins (latency 0) and is invisible to the immune system. However, expression of EBNA-1 (latency 1) is required to tether the episome to the host chromosome to ensure its mitotic segregation during cell division.

Two latent EBV proteins, LMP-1 and LMP-2A have garnered significant interest, both for their role in moving EBV-infected cells to and allowing their survival throughout the germinal center process (reviewed in Ref. [13]) and their possible contribution to lymphomagenesis (reviewed in Refs. [16–18]). LMP-1 acts as a functional homolog of constitutively active CD40 and binds to several intracellular proteins activating the P13k/Akt, NF-kB, Jak/STAT, p38, and ERK MAPK pathways driving B-cell proliferation and survival. LMP-1 also modulates genes involved in cytokine expression (IL-6, IL-10, 1L-8), induces B-cell activation markers (CD23, CD30, and adhesion molecules) and inhibits apoptosis through induction of bcl-2, A20, and c-FLIP. LMP-2A prevents lytic reactivation of EBV in latently infected cells by blocking tyrosine kinase phosphorylation and mimics the nonproliferative tonic signal delivered by the B-cell receptor essential for the survival of all B cells.

Studies of EBV viral load (VL) in the peripheral blood of immunocompetent patients are limited making it difficult to define "normal" VLs during acute infection; adolescents and young adults with infectious mononucleosis have been studied most. In this setting, peak VLs are believed to antecede clinical symptoms, and as many as 10% (more commonly 0.1–1%) of circulating B cells are EBV-infected representing a staggering 50% or more of all memory cells [19]. These numbers decay rapidly (average half-life 7.5 days) through memory B-cell homeostatic mechanisms and/or culling by the CD8 T-cell response [20, 21]. EBV DNA is no longer detectable in plasma by day 15 after onset of symptoms [22]. Within a year there are only 1–60 per million EBV-infected B cells in the peripheral blood of healthy seropositive subjects [20]. This "set point" level remains stable over many years. Whether ongoing seeding of the memory compartment by recurring cycles of infection is required to maintain this equilibrium is uncertain [23]. Using assays that do not detect these low set point levels, Balfour found EBV DNA had cleared from the blood of almost all seroconverting college students by 200 days (median 95 days) [8]. VL data from immunocompetent preadolescent children are sparse [21] and data in infants are limited to studies of African children where infection at a very young age (<6 months) was associated with higher and more persistent VLs [7, 24].

The major site of infectious virus production is the oropharynx. Lytic gene expression is triggered when mB_{Lat}s differentiate into $CD38^{hi}$, $CD10^-$, $CD19^+$, $CD20^{lo}$ plasma cells [25], migrate to mucosal epithelium, and secrete infectious virus into saliva. During the lytic cycle, the EBV genome is linear, and more than 80 viral genes are sequentially expressed under the control of two potent transactivators BZLF1 and BRLF1. These genes play a key role in the switch from latency to the lytic cycle; the epigenetic state of EBV DNA controls this switch (reviewed in Ref. [26]. Lytic genes include two immediate early (IE) proteins that transactivate early gene expression, >30 early (E) proteins that include components of the viral DNA replication complex, and >30 late proteins that include virion structural proteins [1]. The triggers that initiate differentiation and viral replication in mB_{Lat}s in vivo are incompletely understood [26]; whether cognate antigen is required is unknown.

Despite the fact that epithelial cells lack CR2 and constitutively expressed MCH class II, they are an important site for replication and amplification of EBV as a result of either direct infection or infection by contact with lytically infected B cells. Epithelial cell entry does not involve gp42 or HLA II but rather involves the interaction of gH/gL with cell surface integrins [14]. EBV shedding in the saliva of seropositive individuals is continuous and rapid [27]. Quantitative levels in saliva do not directly correlate with numbers of mB_{Lat}s in peripheral blood [8, 27]. EBV-infected cells secrete exosomes, small vesicles important in intercellular communication that may modify the cellular microenvironment, by inhibiting immune cell function or stimulating angiogenesis (reviewed in Ref. [28]). They are found in high levels in human saliva.

Forty-four miRNAs encoded by two regions within the EBV genome, BART and BHRF1, have been described (reviewed in Refs. [29, 30]). Expression is influenced by tissue and latency type. Although viral genes targeted by EBV miRNAs include EBNA-2, LMP-1 and BHRF-1, the majority of viral miRNA target human transcripts including those involved in apoptosis and immune modulation/evasion pathways. Viral miRNAs work in networks with human miRNAs to co-target the same transcripts. The role of viral miRNAs in EBV biology and the pathogenesis of malignancy is the subject of ongoing study.

EBV isolates comprise two major groups, EBV-1 and EBV-2 (also named types A and B), based on the allelic polymorphism of genes encoding EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, and EBNA-LP. EBV-1 predominates throughout the world whereas EBV-2 is equally prevalent in Africa and New Guinea. Further subclassification systems based on polymorphisms in LMP-1, EBNA-2, EBNA-3s, and BZLF1 have been used to examine the impact of strain variability on virus tropism, immune response, and oncogenesis. However, it has been difficult to clearly link EBV genotype variants to specific clinical disease states as reviewed by Hatton [16]. Transmission of EBV is complex with simultaneous transmission of multiple strains, reinfection events and strain evolution. In individual patients, different strains may exist within distinct biologic compartments such as the oral cavity, peripheral blood lymphocytes (PBL), and plasma [31–34].

26.1.1 The Normal Immune Response to Epstein–Barr Virus Infection

The immune response to EBV infection is the result of a complex interaction between humoral and cell-mediated responses, in the face of a significant number of viral immune evasion strategies (as reviewed in Refs. [3, 16, 35]).

There is emerging evidence that natural killer (NK) cells play a significant role in controlling EBV infection (reviewed in Ref. [36]). Phenotypically and functionally distinct NK subsets are found in lymphoid tissue such as the tonsil in comparison to peripheral blood. In the tonsil, less mature $CD56^{bright}$ CD16– cells produce high levels of γ (gamma)-interferon; these cells are believed to reduce or prevent B-cell transformation by EBV. NK cells with intermediate differentiation ($CD56^{dim}$ CD16–) expand in peripheral blood during acute EBV infection and preferentially target cells expressing lytic antigens [37].

Both neutralizing and non-neutralizing antibodies directed against a variety of virally encoded products, many of which persist throughout the lifetime of the host, are generated in response to infection. Neutralizing antibodies may function by limiting the spread of cell-free virus, preventing superinfection with other virus strains, and rendering lytically infected cells susceptible to antibody-dependent cell-mediated cytotoxicity [3]. However, despite the presence of such antibodies, viral replication continues in the oropharynx.

EBV-specific $CD4^+$ and $CD8^+$ CTLs play a particularly crucial role in controlling the infection level during both acute and persistent infection. Even in the peripheral blood of healthy EBV-seropositive subjects, 0.2–2% of the $CD8^+$ population is directed against EBV lytic epitopes with up to 0.5% directed against latent epitopes. During acute infection, there is a highly amplified $CD8^+$ CTL response against lytic antigens that peaks and decays, while the latent antigen-specific response is slower, smaller, and less heavily culled. $CD4^+$ responses are much smaller. Marked hierarchies of immunodominance exist for both lytic and latent antigens with lytic CTL responses focused on IE and E antigens. Lytic and latent antigen $CD8^+$ cells vary in the recovery of migration markers after acute infection. This results in different rates of migration into the tonsil and temporal differences in controlling lytic and latent infections [3], explaining the high levels of virus detected in the oropharynx for >6 months after acute infection.

EBV has also evolved diverse strategies to evade the immune system that include modulation of immune cell function, antigen presentation and apoptotic pathways (reviewed in Refs. [16, 35]). During the lytic cycle, EBV encodes a number of proteins with functional homology to human proteins. BCRF-1 is a homolog of human IL-10, that appears to have retained the immunosuppressive but not immunostimulatory functions of its human counterpart, promoting viral persistence by inhibiting interferon (IFN)-γ (gamma). BARF-1, homologous to colony-stimulating factor 1, acts as a decoy receptor to block the action of this cytokine inhibiting the expression of IFN-α (alpha). EBNA-1 is poorly recognized by CTL because of a long glycine–alanine repeat that inhibits proteasomal processing and MHC class 1 presentation. In addition, a number of proteins expressed during the lytic cycle (BNLF2a, BGLF5, BILF1) inhibit discrete stages of the MHC class I and class II antigen presentation pathways. Latent proteins LMP-1 and LMP-2A perturb apoptotic pathways allowing survival of cells that would normally die in the germinal center process. The lytic antigen, BHFR-1 encodes a homolog of Bcl-2 capable of inhibiting apoptosis induced by multiple stimuli. EBV exosomes and miRNA may also contribute to immune evasion.

Regulatory T cells (T regs) developed during the course of the immune responses provide a counterbalance to immune T-cell activation, protecting the body from pathogen-induced immunopathology. Preliminary evidence exists that T regs are induced during the course of acute EBV infection in both immunocompetent hosts [38] and SOT recipients [39]. They may also play an important role in some EBV-associated malignancies including Hodgkin lymphoma and nasopharyngeal carcinoma [40].

26.1.2 Epstein–Barr Virus Infection After Solid Organ and Hematopoietic Stem Cell Transplantation

Primary EBV infection is very common in the EBV-seronegative patient who receives a transplant from a seropositive individual [41] or HSCT [42], making the donor the most important source of infection in this setting. How the biology of EBV is altered when virus is transmitted though latently infected B cells in the donor organ or hematopoietic stem cells rather than saliva is uncertain. Although a case of transfusion-acquired EBV infection has been described in a liver transplant recipient [43], the risk of transfusion-acquired EBV after transplantation is uncertain but likely low, particularly when blood products are universally leukoreduced. Community-acquired EBV infection is common in young children, adolescents, and young adults [8, 44] and will continue to occur in the transplant setting [45].

Studies of the prevalence/incidence of EBV DNAemia in transplant recipients are difficult to compare because of differences in the characteristics of the populations studied, assays and sample type. In EBV-mismatched (donor positive/recipient negative) SOT patients, EBV DNAemia detection in whole blood ranged from 33% [46, 47] to 100% [41]. In seropositive adults, EBV VL detection in whole blood is highly prevalent and may increase with time after transplant. Reported rates of detection in adult SOT recipients vary from 13 to 72% [48–52]. In a recent study of allogeneic HSCT recipients, the 3-year cumulative incidence of EBV DNAemia measured in plasma was 31.1%; use of antithymocyte globulin (ATG) and intensified conditioning were

identified as risk factors for its occurrence [53]. Umbilical cord blood (UCB) stem cells should not be EBV-infected or transmit EBV. However, EBV DNAemia has been observed in this setting particularly when reduced intensity conditioning (RIC) is used. This likely reflects reactivation of recipient virus. Since PTLD cases in this setting are donor-derived, recipient virus must infect donor cells. The absence of EBV-specific T cell responses in naïve UCB donors combined with T cell depletion can result in rapid uncontrolled proliferation of EBV-infected cells in this setting [54–56].

EBV DNA in the peripheral blood of transplant recipients is strongly cell associated, particularly in the SOT setting, and is usually found exclusively in memory B cells [57, 58] including $IgD^{+}CD27^{+}$ memory B cells that appear to have non-germinal center origins [58]. In some HSCT patients with high VL, EBV DNA has been found in cells lacking B, T, NK, plasma cells, and monocyte markers [59]. In contrast to the latency 0 EBV gene expression observed in the memory B cells of immunocompetent EBV seropositive patients, several investigators found both "growth/latency III" and lytic gene expression surprisingly frequently in the peripheral blood of non-PTLD SOT patients although this expression was transient; these patterns appear to predominate early after transplant [60].

Over 20 years ago several investigators studying SOT and HSCT recipients observed that peak EBV VL measured in PBL or oropharyngeal excretions was higher in primary infection than in reactivation infection and in patients who went on to develop PTLD vs. those who did not; these high levels antedated clinical symptoms. Assays measuring EBV DNA, most commonly using quantitative real-time PCR technology, have been implemented internationally to prevent, diagnose and monitor PTLD treatment, for safety monitoring in clinical trials of new immunosuppressive agents, and for tailoring immunosuppression in individual patients. However, the evidence to support the clinical utility of these assays in many settings is suboptimal (reviewed in Refs. [61–63]).

With more extensive use of VL monitoring a number of investigators observed that many SOT recipients with asymptomatic primary EBV infection or recovering from EBV disease or PTLD have altered EBV "set points" with sustained and elevated EBV VLs in peripheral blood for >6 months, often lasting many years. These patients have been described as having a chronic viral load phenotype (CVLP). Although a study in pediatric thoracic SOT suggests that 45% of CVLP patients developed late onset EBV-positive PTLD at a median follow-up of 7 years [64], the risk appears, in part, to be organ-specific. Intermediate risks have been observed after intestinal SOT [65] with lower risks reported in liver [66] and kidney transplant recipients [41, 67]. However, even among specific allograft types, reported long-term risks vary among centers [66, 68]. Both the prognosis of the CVLP state and its pathogenesis remains uncertain. Recipient HLA [67] and cytokine gene polymorphisms [69] have been suggested as possible risk factors for CVLP. Even in patients with CVLP, EBV DNA is most often B cell-associated [70, 71] although virus was also observed within T cells and monocytes in some patients [71]. In pediatric SOT patients with high VL, Schauer et al. [72] found that up to 30% of EBV-infected cells were aberrant "crippled" or "forbidden" Ig-null cells containing 30–60 genomes/nuclei. Although the results EBV gene expression profiling in CVLP patients [67, 70, 71, 73] are variable, most studies confirm that these patients express a latency 0 pattern with some expressing LMP-2; LMP-1 expression is rare [67, 70, 71]. The number of EBV-specific CD8 cells in these subjects is high [47, 70, 74, 75] but Macedo et al. [74] observed signs of cellular exhaustion (programmed cell death (PD1)+/CD 127− and a decline in γ (gamma)-interferon release) in these cells. In contrast, Moran et al. [75] recently reported a similar frequency of PD1 expression on EBV-specific T cells in CVLP patients vs. those who resolved infection.

Although high levels of antibody to EBV lytic cycle (anti-viral capsid antigen [VCA] IgG and anti-early antigen [EA]) are often seen in SOT and HSCT recipients, serologic responses seriously underestimate infection. SOT patients with the poorest responses or antibody loss (anti-EBNA-1) may be at increased risk of developing PTLD [76, 77]. Passive antibodies from blood products further complicate the interpretation of humoral immune responses in these settings.

26.2 Pathogenesis of Lymphoproliferative Lesions in the Immunosuppressed Host

Although almost all transplant recipients are either infected with EBV pre-transplant or experience primary EBV infection in the early posttransplant period, lymphoproliferative disorders remain a relatively uncommon event. A wide spectrum of PTLD disease states is seen, particularly in the SOT setting. The frequency of specific types of PTLD varies with time after transplant and EBV's role in their pathogenesis may differ [11]. Immunodeficiency, particularly in EBV-specific innate and adaptive responses, is an important contributor to early PTLD but other factors likely participate.

A conceptual framework caller "cancer immunoediting" has been developed to integrate the immune's system's dual role in cancer. It not only suppresses cancer by destroying or inhibiting cancer cells, but also promotes cancer by selecting for cells that escape immune surveillance or creating a tumor microenvironment that facilitates tumor outgrowth [78]. Three phases of cancer immunoediting have been described—elimination, equilibrium, and escape. Early EBV-positive PTLD is an example of failure of the elimination phase; late PTLD may reflect the latter two phases of this process.

Models for EBV-associated lymphomagenesis have been proposed and are summarized in Figure 26-1 [11, 13, 79].

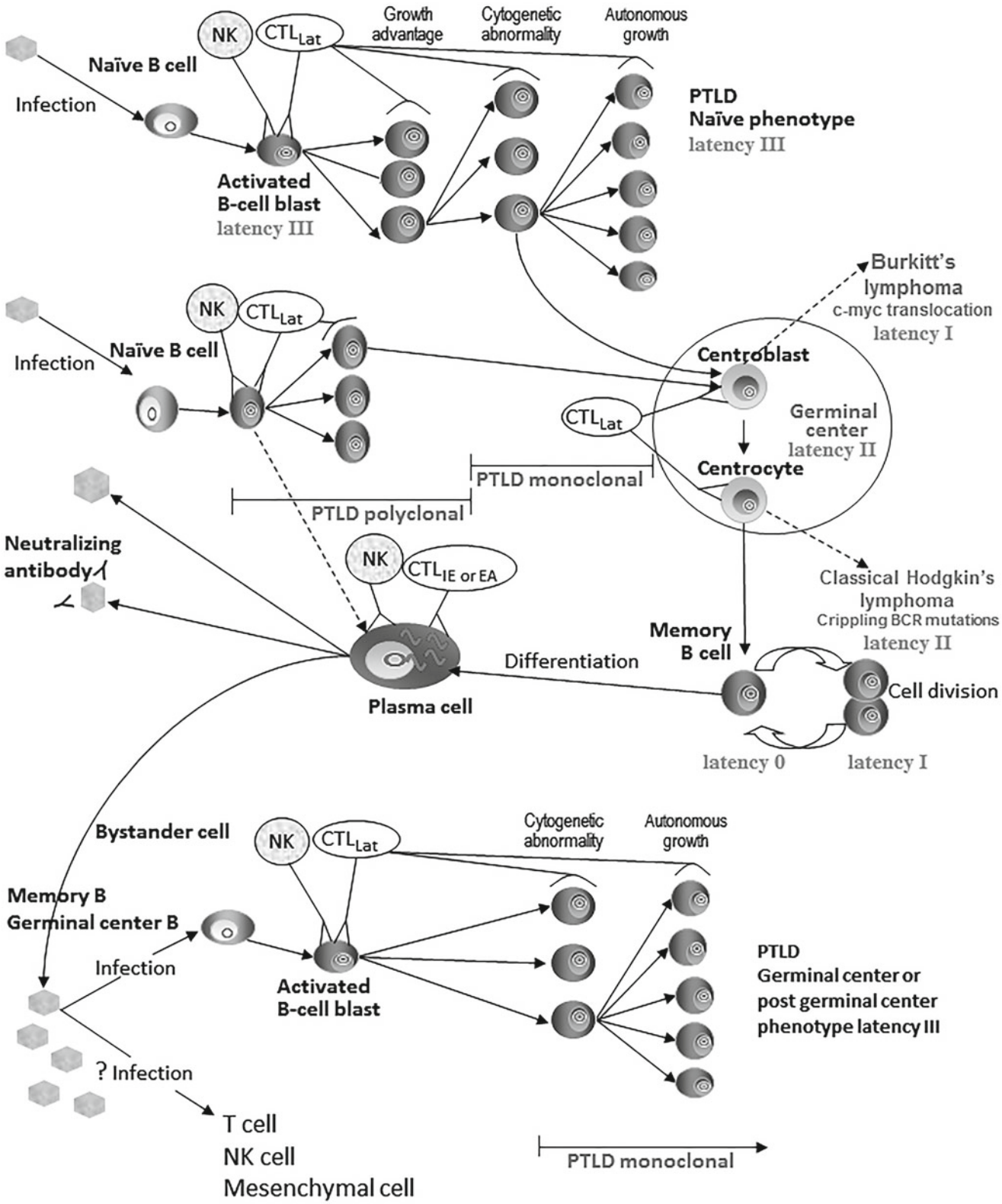

FIGURE 26-1. A model for how Epstein–Barr (EBV) might give rise to posttransplantation lymphoproliferative disorders (PTLDs). Naïve EBV-infected B cells express a repertoire of latent EBV proteins known as "the growth program/latency III," resulting in polyclonal cellular proliferation. Specific clones may have a growth advantage resulting in oligoclonal or monoclonal proliferation. These cells would be cleared by a combination of differentiation into memory cells and killing by EBV-specific cytotoxic T lymphocytes (CTLs). The differentiation to memory cells involves downregulation of latent EBV gene expression from the growth program through a "default program/

When naïve B cells are infected by EBV, they express "the growth program/latency III" resulting in polyclonal cellular proliferation. The local microenvironment may give growth advantage to specific clones resulting in oligoclonal or monoclonal proliferation. The microenvironment encompasses not only immune cells such as T cells, B cells, macrophages; soluble factors such as cytokines and chemokines; but also other cells including endothelial cells, stromal cells, fibroblasts and other viruses (reviewed in Ref. [80]). Variations in the expression of viral genes, host genes transactivated by EBV latent proteins, and virus-induced cytokines such as IL-6 and IL-10 may also drive proliferation. Normally B cells expressing latency III gene products would be cleared by a combination of differentiation into memory cells and destruction by EBV-specific CTLs. In the presence of an impaired immune response, rates of proliferation may exceed rates of clearance and differentiation, leading to clinical presentations with morphology such as plasmacytic hyperplasia and polymorphic PTLD. Genetic or epigenetic abnormalities may also occur, resulting in a more "malignant" form of PTLD. In the transplant setting, particularly with primary infection, delays in the development of the CTL response could result in amplification cycles of new cell infection that continues unabated for significant periods of time and results in the production of enormous virion loads. Chronic B-cell stimulation within the allograft may also increase cycling of cells through the germinal center and possibly lytic reactivation in EBV-infected memory B cells.

Studies of V-gene sequences in PTLD cases suggest that most PTLD is not derived from naïve B cells but have their origin from germinal center and post-germinal center B cells. This dramatically changes theories of pathogenesis. The model has been adjusted by suggesting that de novo EBV infection of these non-naïve "bystander" B cells is an accidental event [12, 79]. When EBV-infected, these cells will express "the growth program," be unable to differentiate out of the cell cycle, and be dependent on the CTL response for destruction. Alternative explanations include the possibility that EBV may be able to impose a memory phenotype without the requirement for a germinal center reaction, or that latently infected germinal center and memory cells fortuitously received signals that caused them to inappropriately turn on the growth program. Failure of a vigorous CTL response to destroy these expanding clones sets the stage for secondary transforming events in the form of genetic and epigenetic alterations in cellular DNA. EBV-positive germinal center B cells may be particularly vulnerable to additional mutation events as this is the site at which somatic hypermutation and class switching occur. Moreover, EBV infection affects both the stability of host genome and DNA damage response pathways [81]. Mutations in viral genes may also alter either critical function or the antigenicity of their protein products, promoting clonal outgrowth.

EBV-1 appears to be more virulent than EBV-2 in promoting lymphoproliferation in vitro and in vivo and PTLD development in vivo. Although epidemiologic and mouse studies suggest that specific strains may be more oncogenic, particularly those with deletion mutations of the carboxy-terminus of LMP-1, or mutations in EBNA3B, a gene that normally might be functioning as a tumor suppressor. However, these data are not conclusive [16, 69]. The post-transplant environment is an ideal setting for the development and selection of these variants because of the opportunity for reinfection events by donor isolates and the high VLs facilitating mutation and recombination events.

How EBV gets into T cells and B cells to cause T and NK cell PTLD is uncertain. It has been suggested that this occurs rarely and only when mature T and B cells are in contact with foci of high EBV replication; ongoing survival of these clones may be dependent on an inflammatory microenvironment [2]. The presence of extremely high levels of infectious virus, particularly during a primary infection, may significantly increase the probability of "accidental" infection of memory and germinal center B cells, T cells, NK cells, and mesenchymal cells that would only very rarely be infected in the immunocompetent host.

FIGURE 26-1. (continued) Latency II" to a transcriptionally silent state in the memory cell (Latency 0). In the presence of an impaired immune response, rates of proliferation may exceed rates of clearance and differentiation, leading to clinical PTLD presentations. Differentiation of EBV-infected B cells into plasma cells results in lytic viral replication, thereby continuing to recruit newly infected cells into the process. NK cells both restrict initial B cell transformation and target lytically infected cells. Mutations may occur during the lymphoproliferation process. If this occurs during the activated B cell blast stage, it results in PTLD with a naïve B phenotype (less common). EBV may provide an anti-apoptotic function to cells lacking functional B cell receptors including those with "crippling mutations" in the germinal-center centrocyte stage, resulting in Hodgkin disease (constitutive expression of the default program), or cells which perhaps because of chronic immune stimulation acquire a c-myc translocation in the germinal-center centroblast stage (Burkitt lymphoma); however, these cells express a virus latency profile similar to dividing memory cells (Latency 1). In the presence of high levels of infectious virus, "bystander" cells, such as germinal-center and memory B cells, that are not normally infected with EBV may become infected. These cells will express the growth program, be unable to differentiate out of the cell cycle, be dependent on the T cell response for destruction, and be vulnerable to mutation events resulting in clinical PTLD (germinal center or post germinal center phenotype, more common). Similarly, accidental infection of T,NK or mesenchymal cells may occur. (Adapted from Thorley-Lawson DA, Gross A. Persistence of the Epstein-Barr virus and the origins of associated lymphomas. N Engl J Med. 2004;350:1328–1337 and Vockerodt M, Ypa LF, Shannon-Lowe C et al. The Epstein-Barr virus and the pathogenesis of lymphoma J Pathol 2015;235:312–322).

Studies of lymphoproliferative disorders in HIV populations suggest that specific types of lymphoma occur at different levels of immunodeficiency, with latency III type lymphoproliferations occurring in the setting of profound immunodeficiency (CD4 T cells <50 cells/ml), Hodgkin lymphoma at intermediate levels (CD4 T cells 50–250 cells/ml) and Burkitt lymphoma in settings with less immunodeficiency (CD4 T cells 250–500 cells/ml) [2]. Whether the same immunodeficiency patterns apply for these types of PTLD is uncertain. EBV's role in Burkitt lymphoma and Hodgkin lymphoma may be to rescue and clonally expand post-germinal center B cells with c-myc mutations (Burkitt lymphoma) or crippling mutations that result in lack of a functional B cell receptor (Hodgkin lymphoma), conditions that would normally have resulted in apoptotic cell death [11]. Increased rates of Hodgkin disease and other HIV-associated lymphoma have been observed in HIV patients with significant initial immunodeficiency within 6 months of receipt of highly active antiretroviral therapy; the presence of immune reconstitution inflammatory syndrome is believed to play a role in lymphoma pathogenesis in this setting [82, 83]. This has not been clearly described in PTLD but might occur in HSCT and SOT settings where there are profound and rapid changes in immunosuppression in the presence of high VLs.

A significant and increasing proportion of PTLD cases late after transplant in the SOT setting do not contain EBV [84]. Suggestions with respect to pathogenesis of these lesions include infection with oncoviruses other than EBV, "hit and run" EBV infection, or coincidental lymphomas identical to those seen in immunocompetent patients [17]. Recently, the importance of chronic inflammation and immune stimulation in lymphomagenesis has been highlighted (reviewed in Refs. [2, 11, 17, 80, 85]). Transplant recipients are particularly vulnerable to this cofactor as a result of chronic graft vs. host disease (GVHD) after HSCT and antibody-mediated rejection as a cause of chronic allograft dysfunction after SOT.

26.3 Epidemiology and Risk Factors for the Development of Posttransplant Lymphoproliferative Disorders

26.3.1 Epidemiology

Immunosuppression in SOT recipients increases the risk of many types of cancer, including those associated with infectious agents, such as Hodgkin and non-Hodgkin lymphomas (NHL) [86–88]. In a large US study, NHLs were among the most common malignancies in transplant recipients, occurring at an incidence of 194 cases per 100,000 person-years (PY) compared to 26 per 100,000 PY in the general population (standardized incidence ratio (SIR) 7.5)) [88]. The overall incidence rate of PTLD after SOT is 1–5% [17], whereas it is lower after HSCT, ranging from 1 to 4% [42, 55, 56, 89]. The incidence of PTLD has likely been underestimated due to limited study follow-up, lack of standardized diagnostic criteria, and the absence of non-monomorphic PTLD in cancer registries.

In kidney and kidney-pancreas transplant recipients, PTLD incidence occurs in a bimodal pattern: an initial peak occurs in the 1st year and a second peak in the 8th to 10th posttransplant years [90–94]. The late peak was not confirmed in non-renal transplants [88]. In sharp contrast, the incidence of PTLD after HSCT peaks at 3 months posttransplant and declines drastically after 6 months [95, 96].

The epidemiology of PTLD has undergone a shift over the past decade. A decrease in the incidence of PTLD has been documented in recipients of renal and non-renal transplants after 2000 even after adjusting for immunosuppressive drugs [90, 97, 98]. The median latency time from transplant to PTLD has increased from 1 to 3 years [99, 100] and EBV-negative PTLD has become more prevalent than EBV-positive PTLD [84]. A decrease in early PTLD (ie. within 1-2 years posttransplant) may account for the overall decline in PTLD incidence; this trend has been attributed to evolving immunosuppressive regimens and EBV VL monitoring with preemptive treatment in seronegative patients [84, 98].

26.4 Risk Factors for the Development of PTLD in Solid Organ Transplantation

Accurate risk stratification for PTLD facilitates the targeting of those at particularly high risk for monitoring and possible preemptive therapy. Although risk factors have been defined, the magnitude of risk attributable to a specific parameter is less clear. Risk factors are often interrelated, requiring rigorous multivariate analysis in large datasets to establish independent effects. Unfortunately, data concerning donor and recipient EBV serostatus, other viral infections, and exposure to immunosuppressive agents is often lacking in registry data. Single center data may provide greater granularity regarding the presence or absence of potential risk factors but lacks statistical power.

26.4.1 Infection with Epstein–Barr Virus and Other Viruses

EBV plays a major pathophysiologic role in the development of almost all early and many late PTLD. Recent analyses have confirmed an observation first made by Ho et al. [101]; patients at risk for primary EBV infection experience a 5- to 18-fold greater incidence of early PTLD compared to seropositive recipients [45, 90, 102–107]. In seropositive individuals, the relative contribution of EBV reactivation vs.

reinfection is unknown. The role of primary EBV infection in late PTLD has been less well studied. However, a case–control study reported a 7.1-fold increased risk of late PTLD in EBV-seronegative adult renal transplant recipients [108] and recent registry analyses suggest increased risk beyond the first posttransplant year [90, 104].

The contribution of other viruses to the risk of PTLD is less clear. Conflicting single-center data exists regarding the impact of CMV mismatching or CMV disease [108–110]. Registry analyses have also reported conflicting results with some reporting no effect [97, 105, 111], a 2.5-fold increase in risk [98] or a modest increase in risk of EBV-negative PTLD only [90]. Missing data is a significant problem, raising concerns that conclusions drawn from registry data may be unreliable. In addition, widespread use of antiviral prophylaxis may have reduced the potential impact of CMV on PTLD risk. Infection with other members of the herpesvirus family may also facilitate the development of PTLD. Human herpesvirus 8 has been linked to serous cavity-based primary effusion lymphoma in recipients of SOT [112].

Hepatitis C virus (HCV) infection may increase the risk of NHL in the general population in certain geographic regions. Liver and cardiac transplant recipients infected with HCV appear to have a 4- to 9.5-fold increased risk of EBV-related PTLD in some [113, 114], but not all studies [110]. Data are lacking regarding the impact of HCV infection in other types of SOT or following HSCT.

26.4.2 The Type of Organ Allografted

The incidence of NHL is highest in intestinal and multivisceral transplant recipients (>10%), followed by lung and heart transplant recipients (3–9%) and lowest for liver and renal recipients (1–3%) (Figure 26-2) [87, 88, 90, 98, 103, 110, 115–117]. In an analysis of the American registry, the SIR of NHL was 6.05 [95% CI 5.59–6.71] for kidney transplant recipients, 7.77 [95% CI 6.99–8.61] for liver recipients, 7.79 [95% CI 6.89–8.79] for heart recipients and 18.73 [95% CI 15.59–22.32] for lung transplant recipients [88]. Registry analyses fail to include intestinal and multivisceral transplant recipients but in a single center analysis of 394 such recipients, the incidence of PTLD was 8% at 6 months, 11% at 1 year, 16% at 5 years, and 27% at 10 years [103]; the majority of cases (71%) occurred during the first posttransplant year with a median time to diagnosis of 5.5 months. The disproportionate number of young pediatric recipients susceptible to primary EBV infection and the relative intensity of immunosuppression may explain the high rates of PTLD in recipients of lung, bowel, and multivisceral transplants. However, it is possible that the amount of lymphoid tissue contained within the allograft and the liberal use of biopsies that detect allograft involvement may contribute.

26.4.3 Recipient Demographics

Most studies have not revealed a gender or racial bias in the risk of PTLD [87, 88, 90, 98, 115, 118]. Recipient age is an important determinant of the risk of PTLD with a U-shaped distribution [87, 88, 98, 115]. In the Collaborative Transplant Study (CTS) registry, those <10 or >60 years of age were at greatest risk [115]. In the American registry, transplant patients aged ≥50 years had the highest incidence of NHL but the SIR was highest in those transplanted under 35 (SIR 46) [88]. A Swedish registry reported a SIR for NHL of 127 for pediatric (age <18) SOT recipients [119]. Similarly, the Australian and New Zealand registry of non-renal transplant recipients reported a SIR for lymphoma of 88.5 in pediatric patients (0–15 years of age) vs. 7.82 in those >15 years of age [87]. High rates of EBV-seronegativity and prolonged immunosuppression confer a high risk to pediatric patients

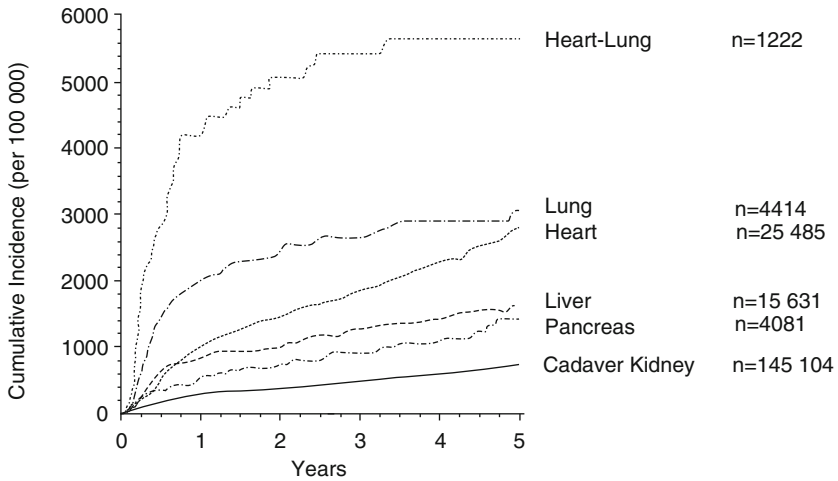

FIGURE 26-2. Five-year incidence of non-Hodgkin lymphomas (NHLs) in organ transplant recipients. Relative risk (RR): heart–lung 239.5, lung 58.6, heart 27.6, liver 29.9, pancreas 34.9, cadaver kidney 12.6 when compared to an age-matched non-transplant population. (From Opelz G, Dohler B. Lymphomas after solid organ transplantation: a Collaborative Transplant Study report. Am J Transplant. 2004;4:222–230, with permission).

[4, 120, 121]. Multi-institutional data in pediatric heart transplantation provides insight as to the complex interaction between recipient age and EBV status [97]. Nearly 25% of EBV-seronegative recipients of EBV-positive donor hearts at age 4–7 years developed some form of PTLD by 5 years posttransplant, although the incidence has declined in the most recent era (2001–2009).

In contrast, older recipient age is a significant risk factor for late PTLD, perhaps due to senescence in immune surveillance exacerbated by immunosuppression [90, 92, 111]. Whether modifications to immunosuppressive protocols in the elderly could ameliorate this risk is unknown. Interestingly, Japanese investigators have described an age-related (senile) EBV-positive lymphoproliferative disorder in immunocompetent individuals that closely resembles PTLD [122].

26.4.4 The Role of Antigenic Stimulation, Cytokine Gene Polymorphisms and HLA

Chronic inflammation may play an important role in the pathogenesis of certain lymphomas as reviewed by Rickinson [2]. The degree of HLA mismatching has not been consistently shown to influence the risk of PTLD, when adjusted for differences in exposure to immunosuppressive medications [90, 91]. However, the alloimmune response may explain the predisposition for PTLD to involve the transplanted organ [103, 115], perhaps facilitating homing, proliferation, and inhibition of apoptosis of EBV-infected recipient B cells. The role of specific HLA antigens on the risk of developing PTLD remains uncertain. Results of studies evaluating the association between HLA alleles and the risk of PTLD have yielded inconsistent findings [84, 106, 123–125], perhaps due to variability in the ethnic makeup of study participants, or the proportion of late or EBV-negative PTLD cases included.

Genetic polymorphisms may determine inter-individual variation in the expression of cytokines, their receptors, or other molecules regulating the response to EBV infection [126]. Results have been inconsistent with respect to the role of low vs. high producer phenotypes for IFN-λ (gamma), TBF-β (beta), or IL-10 on the risk of PTLD [127–129]. Larger studies are required to determine whether cytokine gene polymorphisms or specific HLA alleles determine susceptibility to or the outcome of PTLD.

26.4.5 The Presence and Intensity of the Immunosuppressed State

PTLD was first described as a complication of SOT in the pre-cyclosporine (CsA) era where it presented late and resembled NHL in the immunocompetent population. As immunosuppressive protocols became more potent, complex, and T cell-targeted in nature, a new form of PTLD appeared that is highly associated with EBV infection, occurring earlier after transplant with a tendency to involve the allograft, lymph nodes and gastrointestinal tract. These early lesions are more likely to be polymorphic and respond to reduced immunosuppression. Whether this evolution in the clinical spectrum of disease is the result of widespread use of calcineurin inhibitors (CNI) or reflects the cumulative effects of multidrug protocols is unclear. Fortunately developments in EBV monitoring and preemptive intervention over the last decade have reduced the incidence of this early form of PTLD in SOT.

The lack of randomized controlled trials with sufficient power and long-term follow-up constrains the ability to draw firm conclusions regarding the relative risk of any one agent or regimen. However, anti-lymphocyte antibodies employed for induction or the treatment of acute rejection are associated with a 1.5 to 4-fold increased incidence of early NHL [88, 90, 110, 115]. The subgroup undergoing primary EBV infection is particularly vulnerable [109, 130]. Surprisingly, alemtuzumab, a humanized anti-CD52 monoclonal antibody capable of marked lymphocyte depletion, was not associated with an increased risk of early PTLD in a registry analysis [131]. Whether this is the result of relative sparing of EBV-specific memory T cells or control of the EBV reservoir through concomitant B-cell depletion is unknown.

While the initial experience with CNI suggested an increased incidence of PTLD, lower exposure and improved therapeutic drug monitoring have diminished this risk. There remains controversy as to whether there is still a significant difference in risk between CsA and tacrolimus (TAC) [90, 98, 115]. Amongst the antiproliferative agents, a consistent effect on risk has not been demonstrated for azathioprine vs. mycophenolate [45, 90]. The potential impact of mammalian target of rapamycin (mTOR) inhibitors (sirolimus and everolimus) is of particular interest as these agents have both immunosuppressive and antitumor effects [132, 133]. mTOR inhibitors arrest the cell cycle of EBV-transformed lymphoblastoid cells lines in vitro and fail to protect them from apoptosis. In the SCID mouse model, everolimus reduced the outgrowth of EBV-associated lymphoid tumors. The mTOR pathway is constitutively active in the entire spectrum of PTLD lesions independent of EBV status [134]. In addition, these agents may exert direct effects on EBV replication [135]. Although favorable responses with conversion to an mTOR inhibitor in the setting of PTLD have been described, this may be a reflection of reduced immunosuppressive potency rather than a specific antitumor mechanism. Moreover, some data suggest an increased risk of PTLD in patients receiving mTOR inhibitors [90, 131, 136].

Recent experience with novel immunosuppressive agents highlights the importance of the immune response in controlling EBV infection and its complications. In the initial phase II and phase III trials of belatacept, a fusion protein that selectively blocks costimulation, use of this agent was associated with an increased risk of PTLD (reviewed in Ref. [137]).

In the extended 3-year follow-up of both phase III trials in de novo renal transplant recipients, 1.62% of belatacept-treated patients developed PTLD vs. 0.49% of CsA-treated patients. Pretransplant EBV-seronegativity was a significant risk factor with a ninefold increased risk in those receiving the currently recommended dosing regimen. Of great concern is the observation that 44% of PTLD cases in the belatacept-treated patients involved the central nervous system (CNS). Similarly the early experience with tofacitinib, an oral Janus kinase (JAK) inhibitor identified an increased risk of PTLD in de novo renal transplant recipients receiving the experimental agent (2.35% of 213 patients) vs. none in the CsA control arm [138]. Four of the five cases occurred in EBV-seropositive individuals and in those receiving more intensive dosing.

While it is the cumulative intensity of immunosuppression that impacts the risk of early PTLD, is it less clear whether specific immunosuppressive agents have altered the risk of developing PTLD beyond the first posttransplant year. Rather, the duration of immunosuppression appears to be important. Altering the long-term risk of PTLD may be impossible as long as the success of transplantation hinges on chronic pharmacologic manipulation of the immune response.

26.5 Risk Factors for the Development of PTLD after Hematopoietic Stem Cell Transplantation

PTLD arising in the setting of HSCT is uniquely different from that occurring in SOT recipients (reviewed in Refs. [139, 140]). It is largely an infectious complication in which donor-derived EBV-infected B cells proliferate in the setting of severe and sometimes prolonged T cell dysfunction caused by depletion of EBV-specific CTL in the recipient. The highest risk period is during the first 6 months posttransplant corresponding to the period of most profound immunodeficiency [42, 95]. Lesions are usually of donor origin; recipient-origin disease occurs only in the setting of mixed chimerism or failed engraftment. Although nearly all cases are EBV-associated, EBV serostatus has not been identified as a reproducible risk factor, perhaps due to the lack of documentation of donor and recipient EBV serostatus in many series. However, multivariate analyses from two large centers have reported a 13.6- and 4.6-fold increase in risk of PTLD in EBV-mismatched (donor positive, recipient negative) recipients [42, 141]. The apparent reduction in risk seen in the more recent cohort may reflect the implementation of routine EBV VL monitoring with preemptive rituximab therapy if the EBV VL rose above a certain threshold.

PTLD has been reported to occur in <1% of recipients of allogeneic unmanipulated HSCT from HLA-identical siblings and is extremely rare in the setting of autologous HSCT despite periods of profound immunodeficiency. Patient characteristics such as older age, baseline presence of immunodeficiency, prior splenectomy and repeat HSCT may contribute to the risk [42, 95, 140]. But the use of alternative sources of stem cells from HLA-mismatched related or unrelated donors, reduced intensity conditioning (RIC) protocols with in vivo or ex vivo T-cell depleting regimens, and the more intense prophylaxis and treatment of GVHD has resulted in an evolving epidemiology. The cumulative incidence of PTLD in recipients of HSCT was approximately 4% in recent series, independent of the stem cell source [42, 55, 56]. In a large multi-institutional cohort of 26,901 patients receiving allogeneic HSCT [95] between 1964 and 1994, the use of ex vivo T cell depletion was associated with a RR 3.1 to 9.4 of PTLD depending upon depletion method while the use of in vivo depletion with ATG led to a RR of 3.8. Indeed much of the apparent increase in risk of PTLD in HLA-mismatched (≥2 mismatches) HCST was attributed to the use of these strategies. Methods of ex vivo depletion that specifically targeted T cells or T/NK cells were more strongly associated with early PTLD than those removing both T cells and B cells with a RR of 9.4 vs. 3.1, respectively, when compared to no depletion. Concomitant depletion of donor B cells may reduce the size of the EBV reservoir or number of latently infected donor B cells capable of proliferation in the absence of CTL activity. The more recent use of RIC protocols has proved to be an additional potent risk factor. In a large single center study of 1021 recipients of HSCT between 1996 and 2011 [42], RIC was associated with a 3.25 RR of EBV-PTLD. Of note, all patients developing PTLD in this study had also received ATG. Others have suggested that RIC in combination with either in vivo or ex vivo T cell depletion greatly increases the risk of EBV-reactivation and PTLD [55, 56, 141–143]. While these protocols are less cytotoxic, they are highly immunosuppressive, delaying reconstitution of cellular immunity.

The availability of larger datasets has allowed for robust multivariate analyses capable of addressing the impact of multiple simultaneous risk factors on the incidence of PTLD. A multi-institutional report [95] found that rates of PTLD among patients <50 years of age rose from 0.13% without any additional risk factors, to 1.0% (RR 9.3 on multivariate analysis) with a single risk factor, and 3.9% (RR 45) with two or more risk factors. Patients >50 years old with two or more risk factors had an RR of PTLD that was 237-fold greater than that of younger individuals without risk factors. Similarly the study by Uhlin et al. [42] identified 6 risk factors for the development of EBV-PTLD: HLA-mismatch (RR 5.89), EBV-mismatch (RR 4.97), splenectomy (RR 4.81), use of RIC (RR 3.25), mesenchymal stromal cell treatment (RR 3.05), and acute GVHD grades II–IV (RR 2.65). ATG was not included amongst the variables studied as all patients with PTLD had received this agent. The cumulative risk of EBV-PTLD rose from 0.4% in individuals with one risk factor (ATG included) to 3.0% with two risk factors, to 10.4, 26.5, and 40% with three, four, and five risk factors,

respectively. This approach assists in the identification of specific high-risk groups for targeted surveillance and preemptive intervention.

Less is known about the risk factors for the much less frequent occurrence of late PTLD in HSCT recipients. As in SOT, late PTLD is less likely to be EBER-positive. In the multi-institutional analysis of patients receiving HSCT between 1964 and 1994, chronic GVHD (RR 3.0) and selective T-cell depletion (RR 4.2) were the only significant variables identified [95]. Whether this risk arises from ongoing deficiencies in the CTL response, chronic antigenic stimulation, or the proliferative effects of cytokines produced in the setting of GVHD remains speculative. Treatment of chronic GVHD with immunosuppressive therapy with CsA alone (RR 3.18) or in combination with azathioprine (RR 7.0) further increased risk when compared to the use of steroid and azathioprine, suggesting a similar impact of intensity and duration of pharmacologic immunosuppression as described in SOT.

26.6 Clinical Presentation

The spectrum of the clinical presentation of PTLD ranges from localized lesions to widely disseminated disease, and the histology ranges from an indolent self-limited form of lymphoproliferation to fulminant malignancy. PTLD occurring during the first posttransplant year in recipients of SOT is more likely to involve the allografted organ. In contrast, late PTLD (i.e., beyond the first posttransplant year) is more likely to present with multi-site disease [4]. PTLD may present with a variety of symptoms, making a high index of suspicion valuable. Similar to non-transplant lymphoma, symptoms and signs of PTLD may include B symptoms (i.e., fever, weight loss, and night sweats), painless enlargement of peripheral lymph nodes, and organ dysfunction secondary to infiltration or compression by a mass lesion. Extranodal involvement by PTLD is common, occurring in up to 85% of cases; the best characterized sites are the allografted organ (particularly in kidney and lung recipients), the gastrointestinal (GI) tract and the CNS [90, 99, 100, 144]. In a large cohort of French renal transplant patients, renal allograft PTLD occurred within 2 years, CNS PTLD occurred between 2 and 7 years, and gastrointestinal (GI) tract PTLD predominated after year 7 [90]. Involvement of the thorax, mainly the lung parenchyma or thoracic lymphadenopathy, has been described in recipients of all organ types [145]. Cutaneous involvement occurs in 5–10% of cases [146].

GI tract PTLD occurs in 19–56% of patients [90, 100, 147]; it is particularly common late posttransplant [90] and is most often EBV-negative monomorphic B cell subtype. GI disease can present as ulceration and occult bleeding, bowel obstruction by a mass lesion, or bowel perforation. Significant early mortality can occur both at diagnosis and upon excellent response to therapy, especially with extensive multifocal GI involvement [148]. Posttransplant GI symptoms and unexplained anemia or iron deficiency should be investigated as possible signs of GI PTLD.

PTLD limited to the allograft occurs more commonly early posttransplant and the vast majority involves EBV [99, 115, 149]. Primary involvement in the allograft occurs in 10–30% of cases in renal or liver transplant recipients and more than 50% of cases in lung or intestinal allografts [150, 151]. In contrast, the cardiac allograft appears to be spared from clinically relevant disease. Because of the tendency for early allograft involvement with PTLD, a high index of suspicion must exist for patients presenting with allograft dysfunction, particularly in the presence of known risk factors for PTLD. The detection of EBV with EBER probes by in situ hybridization is useful in differentiating between PTLD and rejection [152]. Disease limited to the allograft was predictive of improved prognosis in some series [115, 144, 153, 154].

Isolated CNS disease (i.e., primary CNS lymphoma) occurs in 5–15% of patients with PTLD on current immunosuppressive protocols; renal allograft recipients appear to have the highest risk of localized CNS disease [90, 115, 155, 156]. CNS involvement may also occur in the setting of disseminated disease where involvement may be isolated to the leptomeninges or cerebrospinal fluid (CSF), without overt lesions in the brain or spinal cord parenchyma. CSF analysis is diagnostic in approximately 50% of such cases. Patients with CNS involvement typically present with an altered mental status or focal neurologic findings. In the largest reported series of primary CNS lymphoma, over 80% of cases occurred late posttransplant and imaging with computed tomography (CT) or magnetic resonance imaging (MRI) revealed a single lesion in 63% [155]. Stereotactic biopsy revealed polymorphic histology in 18% and monomorphic histology in 81%, the vast majority of which were diffuse large B-cell lymphoma (DLBCL); overall 88% were EBV-positive [116]. The prognosis of patients with CNS involvement is poor, with a 3-year overall survival (OS) of 43%, although some patients survive more than 10 years after diagnosis [155].

Recipients of HSCT are at particular risk of developing disseminated PTLD [141, 157]. Such patients usually present within the first few months after transplant with widespread lymphoproliferation and multiorgan failure complicated by the presence of concomitant viral infections or systemic sepsis. The CNS is frequently involved. Prognoses for polyclonal and monoclonal diseases in this setting are similarly poor. Presumably, this aggressive form of PTLD is a reflection of the greater global immunodeficiency that arises from profoundly impaired EBV-specific CTL activity in this setting. If engraftment is delayed and the disease is disseminated, approximately 90% of such patients succumb despite aggressive management. Even if the disease is localized, mortality approaches 30%.

26.7 Other Epstein–Barr Virus Clinical Syndromes

As a result of more widespread use of EBV DNA assays, there has been increasing recognition of clinical syndromes, other than PTLD, that have been attributed to EBV infection after both SOT and HSCT. Infectious mononucleosis syndromes, hepatitis, pneumonitis, gastrointestinal symptoms, and meningoencephalitis have been described [4, 158–160]. EBV infection-associated hematologic signs include leukopenia and thrombocytopenia [4]; a case report of EBV-associated fatal aplastic anemia after bone marrow transplantation has been published [161]. EBV-associated hemophagocytic lymphohistiocytosis, characterized by exaggerated macrophage activation and phagocytosis is seen rarely after SOT or HSCT. Usually observed in the context of primary EBV infection, proliferation of virally driven T cells is important in its pathogenesis [1, 162].

Oral hairy leukoplakia, a nonmalignant lesion of the lateral borders of the tongue, is uncommon after SOT, but spontaneously reversible EBV-positive hairy leukoplakia has been reported in patients early after bone marrow transplantation [163]. This lesion is associated predominately with lytic EBV infection and is usually responsive to antiviral therapy. Sharply circumscribed EBV-positive mucocutaneous ulcers involving the oropharyngeal mucosa, skin, or gastrointestinal tract, previously described in non-transplant patients with immunosenescence or receiving iatrogenic immunosuppression for autoimmune diseases and in a single HSCT recipient [164], have recently been described in the SOT setting [165]. Often misdiagnosed as polymorphic or monomorphic PTLD after SOT, these isolated lesions occur in the absence of a tumor mass; patients have no involvement at other sites and no detectable EBV DNAemia. Pathology is characterized by polymorphous infiltrate and atypical large B cell blasts co-expressing B-cell antigens and CD30, often with Hodgkin/Reed–Sternberg (HRS) cell-like morphology. SOT patients with these ulcers responded to minimal therapeutic intervention including reduction in immunosuppression or rituximab monotherapy [165].

EBV also appears to play a pathophysiologic role in the development of rare EBV-positive smooth muscle tumors after SOT reported most commonly in pediatric recipients. The tumors occur late after transplant at a median of 48 months in a recent literature review [166] and 9.4 years in a single center review of adult kidney transplant recipients in Singapore [167]. These non-hematopoietic proliferations of mesenchymal/stromal origin appear to be multifocal, rather than metastatic when multiple sites are involved and have extranodal presentations similar to PTLD. The liver is a common site of involvement [166]. Tumor cells may be of either donor or recipient origin and have features of type III latency, although LMP-1 expression is rarely observed [166, 168]. Optimal treatment strategies for this type of tumor are unknown. The clinical course may be indolent and complete remission has been described in some cases with immunosuppression reduction alone [166]. Surgical resection (when possible) and reduced immunosuppression appear to result in comparable outcomes; multiorgan involvement, particularly intracranial involvement, is associated with a poorer overall survival [166]. Because these tumors are characterized by Akt/mTOR cell cycle activation, a switch in immunosuppression to mTOR inhibitors has been suggested with some positive responses reported [167–169]. Poor responses have been reported with the use of cytotoxic chemotherapy [168, 169].

26.8 Diagnosis

When PTLD is suspected clinically, a tissue biopsy is required to confirm the diagnosis and to characterize the lesion histologically. Imaging with ultrasound, CT, MRI, or positron emission tomography (PET) scans is used to localize a biopsy target and to establish the stage of disease, but is not considered diagnostic. An excisional biopsy is superior, but a needle biopsy may be acceptable when there is no accessible lymph node or mass. Cytologic analysis of bronchoalveolar washings or CSF can support the diagnosis of PTLD, but is inadequate to subclassify PTLD [61].

26.8.1 Histopathology

Tissue biopsies in suspected cases of PTLD should be reviewed by experienced pathologists and lesions should be classified according to the WHO Classification System updated in 2008 [6]. Table 26-2 summarizes the features of this classification system, which is based largely on cellular lineage and morphology. Ancillary pathological techniques should include routine morphology, immunohistochemistry, and EBER in situ hybridization (Table 26-3); molecular genetic studies of antigen receptor genes may be useful in determining clonality but are not essential for diagnosis [6, 61]. Institutional protocols should be implemented to ensure that the tissue is handled appropriately for ancillary tests.

Most PTLD lesions in SOT recipients are recipient-derived, as confirmed by a recent population-based analysis and review of previous case series [170]. Donor-derived PTLD is more common in recipients of renal and liver transplants, where it tends to occur early after transplantation, to involve the allograft, and to have polymorphic histology [170]. Although intestinal transplant recipients experience a high rate of PTLD involving the allograft, donor derived disease is uncommon [120]. PTLD in HSCT recipients is primarily donor-derived [6]. Molecular profiling has suggested that recipient-derived disease has a phenotype of a B cell in the germinal-center stage of differentiation while

Table 26-2. Categories of posttransplant lymphoproliferative disease (PTLD)

Early lesions[a]
Plasmacytic hyperplasia
Infectious mononucleosis-like lesions
Polymorphic PTLD
Monomorphic PTLD (classify according to lymphoma they resemble)
B cell neoplasms
Diffuse large B cell lymphoma
Burkitt lymphoma
Plasma cell myeloma
Plasmacytoma-like lesion
Other[b]
T cell neoplasms
Peripheral T cell lymphoma, NOS
Hepatosplenic T cell lymphoma
Other[b]
Classical Hodgkin lymphoma-type PTLD

Reprinted from Swerdlow SH, Webber SA, Chadburn A, et al. Posttransplant lymphoproliferative disorders. In: Swerdlow SH, Campo E, Harris NL, et al., eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Geneva: WHO Press; pp. 343–344, copyright 2008.

[a]Some mass-like lesions in the posttransplant setting may have the morphologic appearance of florid follicular hyperplasia or other marked but non-IM-like lymphoid hyperplasias.

[b]Indolent small B cell lymphomas arising in transplant recipients are not included among the PTLD.

Table 26-3. Ancillary pathological studies useful for the categorization of PTLD

Immunophenotype[a]
Presence of EBV (EBER in situ hybridization)[a,b]
Clonality (immunoglobulin genes, T cell receptor, EBV)
Genetic/cytogenetic studies (chromosome or oncogene abnormalities)
Donor-vs.-recipient origin
Therapy-dependent markers (expression of CD20, cytotoxic T cell epitopes)

[a]Studies essential for classification.

[b]Immunohistochemical analysis for EBV LMP-1 is specific but less useful.

donor-derived PTLD are more consistent with a mature post-germinal center phenotype [171]; this suggests antigen stimulation may be involved in the pathogenesis of donor-derived disease when antigen-selected cells acquire genetic lesions as they expand in the recipient environment.

26.8.1.1 Early Lesions

Early PTLD lesions are composed of mixed populations of small lymphocytes, plasma cells, and immunoblasts with no cytologic atypia and preserved tissue architecture. This category includes lesions with features of plasmacytic hyperplasia and infectious mononucleosis. Most occur within a short time after transplant and are related to EBV infection [116].

26.8.1.2 Polymorphic PTLD

When lesions demonstrate the full spectrum of B cell maturation, efface the architecture of lymph nodes, or form destructive extranodal masses that do not fulfill criteria for recognized lymphoma types in the immunocompetent host, the lesions are classified as polymorphic PTLD. They are composed of a heterogeneous population of cells with variable atypia, such as atypical immunoblasts and Reed–Sternberg-like cells, and variable plasmacytic differentiation. The majority are monoclonal and some harbor genetic changes. Most of the cases that occur early posttransplant are positive for EBV [17, 116].

26.8.1.3 Monomorphic PTLD

Monomorphic PTLDs are neoplastic lymphoproliferations with sufficient architectural and cytologic atypia to constitute lymphomas, and they are histologically indistinguishable from corresponding pathologic entities in immunocompetent patients [6]. Most monomorphic PTLDs are of B-cell origin, and over 80% of these are DLBCLs; other subtypes include plasmablastic lymphoma, plasma cell neoplasms, and Burkitt lymphoma. The remaining monomorphic PTLDs originate from T or NK cells. Despite the morphologic similarities between monomorphic PTLD and their non-transplant counterparts, recent studies have identified distinct molecular features (reviewed in Ref. [17]).

PTLD of T- or NK-cell origin comprises 5–15% of monomorphic PTLD. The most common subtypes are peripheral T-cell lymphoma (PTCL) NOS and hepatosplenic T-cell lymphoma (HSTCL). Several case series characterize these lesions as occurring late posttransplant (median over 5 years), and primarily involving extranodal sites [172, 173]. Monomorphic T-cell PTLD carries a poor prognosis [154], and HSTCL has particularly poor outcomes with median OS of 4 months [173]. Other predictors of poor survival include bone marrow, CNS or graft involvement [173]. EBV is only detectable in about a third of cases, including all extranodal NK/T cell lymphomas, about half of PTCL, and almost no HSTCLs, but its presence predicts a more favorable prognosis [172]. The pathogenesis of EBV-associated T-cell PTLD is uncertain; EBV episomal monoclonality suggests that it is not simply due to bystander infection. In Japan, T-cell PTLD is also frequently associated with HTLV-1 [174]; its role in other geographic regions is unknown. Disease is often aggressive and refractory to reduction in immunosuppression and therapy, with a median survival of 6 months [172, 174].

Plasma cell neoplasms, including multiple myeloma and extramedullary plasmacytomas, were 1.8 times more likely to occur in SOT recipients than the general population in a recent study [175], but they comprise only 10% of PTLD [176]. Patients with plasmacytoma-like PTLD involving

solitary extranodal masses have a particularly good prognosis [177]. The literature also includes several cases of mucosa-associated lymphoid tissue (MALT) lymphomas involving the stomach or parotid gland in SOT recipients [178], but the most recent WHO classification system does not include these indolent lesions among PTLD subtypes [6].

26.8.2 Hodgkin Lymphoma Type PTLD

Classical Hodgkin lymphoma type PTLD is the least common subtype of PTLD. Incidence of Hodgkin lymphoma after SOT does not exceed the population rate in the first 2 years, but rises steadily thereafter, reaching 13.8 cases per 100,000 at 8–10 years posttransplant (i.e., SIR 4.1) [179]. Males and patients younger than 20 at transplant appear to carry two and four times the risk, respectively, but a predilection for renal recipients was not confirmed, contrary to early reports [180]. It is important to distinguish classical Hodgkin type PTLD from Hodgkin lymphoma-like PTLD histologically. Reed–Sternberg cells in Hodgkin lymphoma-like PTLD are EBV-positive, CD15-negative, CD45-positive, and CD20-positive, and can be accompanied by small or intermediate-size EBV-positive lymphoid cells [6]. It is best categorized as polymorphic or monomorphic PTLD. In contrast, classical Hodgkin type PTLD meets the criteria used for this diagnosis in immunocompetent patients. It appears to respond best to conventional management for Hodgkin lymphoma, namely chemotherapy [148, 181]. Despite morphologic similarities, outcomes for Hodgkin lymphoma type PTLD are inferior to those in non-transplant patients [182]. Interestingly, there are several reports of patients developing classic Hodgkin disease several years after more typical PTLD [183]. Epidemiologic population-based studies in Scandinavia and England have clearly identified symptomatic infectious mononucleosis as a risk factor for EBV-positive Hodgkin lymphoma in immunocompetent patients [184, 185]. Whether EBV disease or early PTLD after primary EBV infection increases the risk for future Hodgkin disease in the transplant setting in a similar way requires further study.

26.8.3 Staging

Staging is important to provide a standardized reference system for the relationship of tumor burden to outcome. At the very minimum, staging should document the precise anatomic location and size of lesions. While the Ann Arbor staging system is typically used for adults [186], other staging approaches such as the Murphy system have been used in children [187]. Although clinical assessment, including inquiry for symptoms and performance status as well as physical examination, remains important, the sensitivity for identifying areas of disease involvement is poor. Therefore, imaging of the chest, abdomen, and pelvis is recommended at baseline [61, 188].

There are no high quality studies to establish the ideal imaging technique for patients with PTLD. Recently published guidelines recommend PET-CT scans as the modality of choice for fluorodeoxyglucose (FDG)-avid lymphomas, specifically Hodgkin lymphoma and aggressive subtypes of NHL, for initial staging and response evaluation in the non-transplant setting [188]. PET-CT scans improve accuracy of staging when compared to CT scans and results lead to a stage change in 10–30%, although no impact on overall outcomes has been demonstrated [189].

Several small case series have confirmed that PTLD lesions are FDG-avid and that PET-CT is an effective imaging modality in staging PTLD patients, with a higher sensitivity than CT for detecting extranodal disease [190–194]. A retrospective review of 150 SOT or HSCT recipients who had PET scans either for biopsy-proven or suspected PTLD found an overall sensitivity and specificity of 89%, and a positive and negative predictive value of 91 and 87%, respectively, demonstrating an excellent ability to differentiate PTLD from non-malignant disease [195]. However, some subtypes of PTLD, such as monomorphic T-cell lymphomas, may not be FDG-avid, necessitating CT as an alternate staging modality. In addition to staging, PET-CT is more accurate than CT in assessing treatment response in non-transplant lymphomas as it can better distinguish residual viable tumor from necrosis or fibrosis [188]. Whether this is also true in the setting of PTLD requires further study, but preliminary data are encouraging [190]. Availability of PET-CT may be limited in some regions, and CT should be the default modality in these cases.

Bone marrow biopsy has traditionally been recommended for routine staging in non-transplant lymphomas, but no data is available on its utility in PTLD. Bone marrow involvement by PTLD represents a negative prognostic factor in some series [100, 173, 196]. Recent guidelines for non-transplant lymphomas cite bone marrow signal on PET-CT as a valid surrogate for bone marrow biopsy [188].

Assessment for CNS involvement is not routinely performed at diagnosis in non-transplant lymphoma [188], and no data is available on this practice in PTLD [61]. In the presence of neurologic symptoms, MRI brain is an excellent imaging modality for detecting CNS PTLD [197].

26.8.4 Epstein–Barr Virus Viral Load

Although EBV VL testing is used extensively to support EBV disease and PTLD diagnosis, the interpretation of results in this setting is problematic. A major problem is the lack of assay standardization. Studies have documented large variability in assay sensitivity and quantitative results reported when the same sample is tested [198, 199]. This variability is exacerbated by relatively few available

commercial assays and the large number of laboratory-developed assays used. In 2011, the WHO approved an international reference standard (IS) for EBV DNA to be used as a common assay calibrator [200]. Although it is hoped that this will improve harmonization of assay results, other factors such as extraction methods, gene targets, and instrument platforms also influence results [201]. Until the impact of the WHO IS on reported result variability has been evaluated, inter-institutional comparisons require formal cross-referencing of assay results. This also means that, currently, it is not possible to recommend specific qualitative or quantitative EBV DNA measurements to serve as globally applied triggers for preemptive intervention, PTLD diagnosis, or treatment end-points.

The optimal matrix for EBV VL measurement (i.e., whole blood vs. plasma) also remains uncertain. Measurement of EBV DNA in whole blood and lymphocytes appears to be more sensitive than measurement in plasma for the early detection of EBV reactivation, suggesting that whole blood may be a better matrix for use in preemptive monitoring strategies [71, 202]. Recent studies in whole blood showed a close correlation between copies/ml and copies/μg DNA with similar dynamic trending in patients using both reporting formats; normalization to cell number or genomic DNA in cellular specimens may be unnecessary [202, 203]. Although EBV generally becomes detectable in plasma as VL rises in matched whole blood samples, the quantitative results reported in plasma have suboptimal correlation with those reported in whole blood or lymphocytes, particularly in HSCT [71, 202].

Studies evaluating EBV VL as a diagnostic test for PTLD in patients with symptoms or signs but with no previous VL monitoring are limited. In lower risk adult SOT patients with unknown pre-transplant serology, qualitative detection of EBV DNA in plasma was highly specific for EBV-positive PTLD but lacked sensitivity, missing some cases of localized EBV-positive PTLD and all EBV-negative PTLD, the predominant form of late PTLD in adults [52]. Whether these data can be extrapolated to high-risk patients or to the broader transplant population is uncertain. Some investigators have suggested that plasma has better specificity for "high load" and may be the preferred specimen when used for the diagnosis and monitoring of therapeutic response of PTLD but studies to date have been small and require further validation [52, 159, 204, 205].

PTLD may be compartmentalized in only CNS or the allograft. EBV DNA detection in CSF in the absence of detection in peripheral blood has been reported in HSCT patients with CNS PTLD [206, 207] and in bronchoalveolar lavage (BAL) fluid in pediatric thoracic transplant recipients with lung PTLD [208]. However, EBV DNA, sometimes at high levels, can be frequently detected in BAL fluid of adult transplant recipients without PTLD as well as in immunocompetent patients [209, 210]. There has only been limited evaluation of EBV DNA measurement in CSF for the diagnosis of CNS lymphoma and CNS disease in transplant recipients [160, 211]. The gold standard of biopsy-proven disease is lacking in many patients in these studies, making interpretation difficult. Additional validation of EBV DNA measurement in CSF and BAL fluid, including assessment of quantitative levels that might improve specificity and positive predictive values for EBV disease or PTLD, is required.

26.9 Strategies for the Prevention of Posttransplant Lymphoproliferative Disorder

Given the absence of reliably effective therapy for all stages or forms of PTLD after SOT and HSCT, strategies for prevention in high-risk recipients have emerged as a major focus for PTLD management. It is important to identify these patients prior to transplantation. EBV serostatus should be determined in all donors and recipients. Wherever appropriate, particularly in EBV-mismatched recipients, immunosuppression should be minimized and the risk vs. benefit of using immunosuppressive agents that selectively deplete T cells relative to B cells as induction or rejection therapy should be carefully evaluated.

Two major approaches have been used for PTLD prevention. The first strategy, "universal prophylaxis," involves the administration of antiviral drugs, immunoglobulin or adoptive immunotherapy to all patients considered to be at increased risk for PTLD, usually beginning shortly after transplant. The second, termed a "preemptive" strategy, combines serial monitoring of peripheral blood EBV DNA levels with interventions that might lower the risk of PTLD. Such interventions would be triggered by EBV DNA levels that are predictive of PTLD risk, but occur before the onset of clinical disease.

26.9.1 Universal Prophylaxis Using Antiviral Drugs or Immunoglobulin

Antiviral drugs such as acyclovir, ganciclovir, foscarnet, and cidofovir [212] as well as the newer agents, brincidofovir [213] and maribavir [214], under development for CMV disease, have in vitro activity against lytic EBV replication but have no activity against EBV infection in its latent form. Acyclovir and valacyclovir treatment in immunocompetent patients with infectious mononucleosis significantly reduces or eliminates oropharyngeal EBV shedding [215, 216]. In seropositive immunocompetent patients remote from acute infection, although 1 month of acyclovir therapy had no effect on EBV VL in peripheral blood [217], a 1-year course of low dose acyclovir did result in a modest reduction.

The use of antiviral drugs for universal prophylaxis has not been evaluated in randomized controlled trials; their role

in PTLD prevention remains controversial. Cases of PTLD have occurred in patients receiving both acyclovir and ganciclovir prophylaxis. When compared to historical controls some studies evaluating antiviral prophylaxis demonstrated a reduction in PTLD incidence; others observed no effect as reviewed by Funch [218]. A multicenter case-control study found that the risk of PTLD was reduced in kidney transplant patients receiving acyclovir or ganciclovir for prophylaxis or treatment, with protective effects most marked in the first transplant year, increasing with duration of therapy, and greater for ganciclovir than acyclovir [218]. In contrast, the use of acyclovir or ganciclovir CMV prophylaxis did not impact PTLD incidence in a registry study of recipients of deceased donor kidney transplants [219].

Since primary, often donor-transmitted EBV is a major PTLD risk factor after SOT, reduction in early infections may extrapolate to reduced PTLD risk. A reduction in primary EBV infection was observed in a recent analysis of ganciclovir/valganciclovir prophylaxis vs. no prophylaxis in a small non-randomized study of mismatched pediatric kidney SOT recipients [41]. Intriguingly, a pilot study in a similar population suggested that 2 weeks of valganciclovir treatment of organ donors may reduce recipient PTLD risk [220]. These preliminary observations require further evaluation, ideally in randomized controlled trials.

The utility of passive administration of EBV neutralizing antibodies (via IVIG) as a prophylactic strategy also remains unclear. Two prospective randomized placebo-controlled trials, one using CMV-IVIG prophylaxis in EBV-seronegative pediatric liver recipients [221], and the second examining the benefit of IVIG when added to ganciclovir in EBV-mismatched adult and pediatric SOT recipients [222] failed to observe a reduction in PTLD rates in patients receiving the immunoglobulin products. The latter study also did not observe an impact of IVIG on EBV VLs in peripheral blood. In contrast, results of an epidemiologic study by the CTS registry added to the uncertainty by reporting that the use of CMV-IVIG reduced the incidence of NHL in kidney transplant recipients but only in the first posttransplant year [219].

26.9.2 Prophylactic Adoptive Immunotherapy

Adoptive transfer of T-cell immunity is an attractive strategy to facilitate the restoration of EBV-specific CTL responses. This is particularly true in HSCT where most lesions are of donor origin and express the most immunostimulatory EBV latent cycle antigens. The infusion of unfractionated lymphocytes from their EBV-seropositive donors (i.e., donor lymphocyte infusion (DLI)) has been shown to be effective in HSCT recipients but is associated with a significant risk of developing GVHD. A refinement of this approach is the infusion of polyclonal, EBV-specific, predominately $CD8^+$ CTL cell lines prepared from donor leukocytes (reviewed in Refs. [5, 223] in the setting of HSCT or recipient leukocytes in the setting of SOT. In HSCT recipients, this approach is safe and effective, with functional EBV-specific CTL responses persisting for up to 9 years [224]. Moreover, this strategy provides cellular immunity targeted at EBV-disease while avoiding the unwanted responses of GVHD. However, in SOT patients who are EBV-seropositive prior to transplant, the low incidence of PTLD renders the routine production of autologous cloned, EBV-specific CTL impractical and costly. The patients at highest risk for PTLD, EBV-seronegative recipients, do not have autologous CTLs from which to develop cell lines. To generate a CTL response from naïve T cells ex vivo has proved challenging. Moreover infused CTL have limited persistence in the SOT setting, perhaps due to ongoing pharmacologic immunosuppression. The production of EBV-specific CTL is time-consuming (usually 2–3 months) and expensive, limiting widespread clinical applicability. Several groups have focused on developing rapid manufacturing strategies to overcome these obstacles (reviewed in Ref. [5]; these include rapid capture techniques to identify polyclonal EBV-CTL, the use of genetically modified dendritic cells as stimulator cells, or the use of overlapping EBV peptide pools as a source of antigen. There is also great interest in the development of banks of cloned, third-party EBV-specific CTL [225]. This would allow for an immediately accessible source of EBV-CTL, particularly for patients with rapidly progressive disease or those recipients of transplants from EBV-seronegative donors. Other notable developments that might improve the in vivo efficacy of adoptive therapy include genetic manipulation to create CNI-resistant CTL [226] and the availability of banked third-party virus-specific T cells with simultaneous activity against other viruses (CMV and adenovirus) that can also cause serious disease in both SOT and HSCT recipients [227].

Adoptive immunotherapy for prevention of PTLD has been given either to all high-risk patients, or preemptively in response to EBV DNAemia (reviewed in Refs. [223, 225]. These approaches have been most extensively evaluated in patients receiving HSCT from matched unrelated donors or mismatched family members in the context of T-cell depletion protocols, in those with a previous history of EBV-PTLD, or who had an underlying immunodeficiency where the incidence of EBV-PTLD may be as high as 11% [224]. Only one case of PTLD was observed in 108 HSCT and 21 SOT high-risk patients who received EBV-CTL prophylactically; that patient had received a CTL line that lacked strong EBV-specificity [223, 225]. None of the HSCT recipients developed de novo GVHD following CTL infusion.

26.9.3 Preemptive Management

The preemptive approach has evolved as the preferred PTLD prevention strategy and is recommended in international guidelines in EBV-mismatched (donor seropositive/recipient seronegative) SOT recipients and in high-risk HSCT recipients [4, 61, 62, 228]. European guidelines for SOT also

recommend use of a preemptive strategy for all lung and intestinal allograft recipients and all patients receiving treatment for acute rejection [62]. However, the evidence to support this strategy in adult EBV-seropositive recipients in these cohorts is weaker. These strategies are not recommended for lower risk populations where the risk vs. benefit is uncertain.

26.9.3.1 Monitoring Algorithms and Trigger Points for Intervention

In addition to issues related to EBV assay standardization and choice of specimen type, optimal monitoring algorithms have not yet been defined. Monitoring algorithms are most cost-effective when used during the posttransplant period of highest risk, defined as 3–6 months in HSCT and at least 1 year in SOT. Measurement of CD4+ cells as a marker of immune reconstitution in HSCT [229, 230] and SOT recipients [231] may better tailor the monitoring period in individual patients; patients with GVHD may require longer monitoring [229, 230]. VL can rise very quickly, particularly in severely immunosuppressed HSCT patients. In a recent HSCT study, the median time to EBV disease from onset of EBV DNAemia was 7 days (range 0–17 days) [53]. EBV DNA kinetics may be as important as absolute quantitative values for triggering interventions [4, 53, 61, 62]. Monitoring more frequently than the currently recommended once weekly may be indicated in the highest risk HSCT subgroups [53]. Less frequent monitoring at intervals varying from weekly to monthly has been recommended in SOT recipients [4, 61, 62].

There are very few natural history studies relating EBV DNAemia levels to PTLD events where clinicians were blinded to results. Interpretation from non-blinded studies is complicated by both the heterogeneity of the populations studied, as well as the non-standardized assays and sample types used (reviewed in Ref. [63]). As a result of these factors, defining optimal trigger points for preemptive intervention is difficult [232, 233]. A recent multicenter analysis of HSCT PTLD cases highlights this problem. EBV DNA levels at the time of PTLD diagnosis were below two commonly used trigger points for preemptive intervention in 45 and 23% of cases, respectively [157]. Although EBV DNA detection has an excellent negative predictive value for early EBV+ PTLD after both SOT and HSCT, preemptive trigger points, often center-specific, have a positive predictive value of only 28–65% in SOT [63] and 25–50% in HSCT [229, 234].

To improve the specificity of high VL for PTLD prediction, combining VL with testing of additional biomarkers has been explored. The most promising is the quantitation of EBV-specific CTLs using tetramers, ELISPOT or flow cytometry-based intracellular cytokine staining [235–238]. However, there are few commercially available CTL assays and current assays are costly, complex, not very rapid, and not standardized [239]. Additional biomarkers proposed for risk stratification that require additional evaluation include assessment of global immunosuppression using commercial ATP-release assays [74], and measurement of IL-6 and 1L-10 [240, 241], soluble CD30 [242], CXCL13 [243], or serum free light chain levels [244, 245].

26.9.3.2 Preemptive Interventions in SOT

Effective preemptive strategies require that the intervention lowers PTLD risk without significant adverse events including graft rejection [5, 246, 247]. Although preemptive interventions are recommended for high risk, predominantly EBV-mismatched SOT patients early after transplant during the course of acute EBV infection, there is no data to support their use in patients with established CVLP later after transplant. Whether any intervention strategy described has any long-term effect on either VL kinetics or PTLD risk in CVLP patients remains uncertain.

Reduction in immunosuppression (RIS) remains the mainstay of preemptive treatment in SOT [248]. However, the process for best achieving this is uncertain; suggestions are outlined in international guidelines [62, 249]. Some advocate a change in immunosuppression to mTOR inhibitors [248]. Although antiviral drugs (ganciclovir/valganciclovir) or intravenous immunoglobulin (IVIG) are sometimes given, often simultaneously with RIS [250, 251], the added benefit of these agents is uncertain [252]. Rituximab, a chimeric antibody directed against the CD20 antigen expressed on B cells, has been used less frequently as a preemptive intervention in SOT than in HSCT [248]. The risks vs. benefits of using rituximab when compared to RIS alone in SOT are unknown. Benefits may be greater in the setting of thoracic transplantation where the consequences of acute rejection following RIS may be higher. Experiences with rituximab have been reported in heart transplant recipients failing RIS [253] and EBV-mismatched kidney transplant patients given rituximab simultaneously with RIS [137]. Preliminary data from intestinal SOT [254] suggest that incorporating rituximab into routine induction regimens in high-risk patients might also be considered as an alternate strategy to reduce risk.

26.9.3.3 Preemptive Interventions in HSCT

RIS is recommended as an initial preemptive intervention in HSCT when possible [228]. However, RIS often fails to alter VL kinetics in the short term particularly very early after HSCT when viral load is rising quickly, the patient is profoundly immunosuppressed and immune reconstitution will take some time [140]. In addition, the risk vs. benefits of reduced immunosuppression, particularly in patients with preexisting GVHD, should be considered. The routine use of sirolimus for GVHD prophylaxis in HSCT has been suggested but the impact of this strategy in reducing the incidence of PTLD has not been evaluated [246]. The utility of using anti-viral drugs alone for preemption is uncertain. Liu

found that only 2 of 16 HSCT patients who received antiviral drugs as the only preemptive intervention responded vs. a complete response (CR) rate of 45.8% when combined with reduced immunosuppression [53].

Because RIS and antiviral therapy have high failure rates as interventions, rituximab therapy has become a common initial preemptive intervention in HSCT [5, 246]. Although a standard weekly dose of 375 mg/m^2 is recommended, optimal dosing and number of doses required remains uncertain [233]. Incorporating rituximab into HSCT conditioning regimens in high-risk patients has also been employed as an alternate strategy to reduce PTLD risk [255]. Safety concerns associated with preemptive rituximab include possible excess bacterial infection due to delayed immune reconstitution, higher non-relapse mortality in HSCT recipients [256] and CD20 escape mutants causing PTLD [257]. Significant side effects of rituximab observed in other settings include infusion reactions, B cell lymphopenia sometimes associated with hypogammaglobulinemia, CMV and hepatitis B reactivation, and progressive multifocal leukoencephalopathy [4]. Ongoing safety monitoring and improved targeting of rituximab therapy to the highest risk patients is warranted. Although adoptive immunotherapy for preemptive intervention is attractive as an alternative to rituximab or in cases of rituximab failure in HSCT, widespread accessibility to this therapy remains problematic [223, 225].

26.9.3.4 *Efficacy of Preemptive Therapy*

There are neither randomized controlled trials comparing preemptive strategies vs. placebo nor specific interventions to each other. However, there is an increasing body of evidence (primarily single center reports) that suggests that preemptive strategies have reduced the incidence of early PTLD in high-risk patients when compared to historical cohorts after both SOT and HSCT (reviewed in Refs. [4, 5, 62, 249]).

26.10 Treatment of Posttransplant Lymphoproliferative Disorders

The approach to treating established PTLD in both SOT and HSCT involves restoring the immune response to EBV and eradicating the proliferating lymphocytes while maintaining graft function. Patients are vulnerable to toxicity in both arms of this approach, and therefore must be monitored closely with involvement of multiple disciplines, including the transplant physician, infectious diseases specialist, and hematologist/oncologist [249]. The mainstays of therapy for PTLD in SOT include reduction of immune suppression, rituximab, and chemotherapy. Adequately powered randomized controlled trials of therapeutic interventions for established PTLD have not been performed primarily due to its rarity. Therefore, recommendations for the management of PTLD in SOT have been solely based on small phase II clinical trials, retrospective case series, and expert opinion [148, 249]. Recently, however, results from the largest international multicenter phase II clinical trial ever conducted in PTLD were published, laying the groundwork for high-quality clinical evidence for treatment of PTLD in SOT (discussed in Sect. 26.10.6) [258] (Table 26-4).

Table 26-4. Options for the treatment of posttransplant lymphoproliferative disorder

Increase the cytotoxic T cell (CTL) response
Reduction or withdrawal of immunosuppression
Adoptive immunotherapy with autologous EBV-specific CTL
Adoptive immunotherapy with partially HLA-matched allogeneic CTL
Antiviral strategies
Ganciclovir/valganciclovir, acyclovir/valganciclovir
Foscarnet, cidofovir
Histone deacetylase inhibitor combined with ganciclovir
Intravenous immunoglobulin (IVIG)
Cytokine therapy
Interferon-α
Anti-IL-6 monoclonal antibodies
Tumor debulking
Anti-B cell monoclonal antibody therapy (rituximab)
Cytotoxic chemotherapy
Surgical resection
Local irradiation

IL interleukin.

Therapeutic options are limited in HSCT patients with established PTLD. Reducing immune suppression is not always feasible or effective, whereas adoptive immunotherapy is highly effective but unavailable at most centers. Rituximab monotherapy is relied upon, but approximately 30% fail [157]. Published outcomes of therapy in this setting are limited to case series and retrospective reviews (reviewed in Refs. [5, 259]).

26.10.1 Reduction of Immunosuppression (RIS)

Reduction or discontinuation of immunosuppression is the most important initial step in the management of PTLD after SOT [4] but may have less utility in early PTLD after HSCT where regeneration of cellular immunity is delayed and the risk of developing GVHD is significant [246]. In SOT recipients, response rates to this strategy have ranged from 0 to 73%. A retrospective study reported that 45% of 67 patients responded to RIS, an effect not limited to those with polyclonal or EBV-associated disease [260]. A favorable response was less likely in older recipients, those with bulky disease, or advanced stage; there was a 17% rate of relapse in complete responders and a 32% rate of acute rejection. In contrast, others have reported CR rates of <10% to RIS alone [261, 262]. RIS are unlikely to have a significant impact on the course of some forms of PTLD such as late NHL, multiple myeloma, and most T-cell lymphomas.

There is no consensus on which agent should preferentially be reduced or the duration of the reduction. A recommended strategy is to discontinue any antiproliferative agents and

reduce the dose of CNI by 25–50% while maintaining the dose of steroid [62, 249]. Recent studies suggest that maintaining a low dose of CNI rather than discontinuing it completely is associated with better long-term graft survival without impairing the response to other PTLD treatments [262, 263]. Responses are typically seen in 2–4 weeks; waiting up to 6 weeks in stable patients without progressive disease may be warranted given that the median time to failure in non-responders was 45 days [260]. The utility of conversion to an mTOR inhibitor after PTLD is diagnosed is uncertain but warrants further investigation.

26.10.2 Adoptive Immunotherapy

In some settings, reduction of immunosuppression is not possible. Papadopoulos et al. [264] initially described dramatic responses to infusions of unfractionated donor leukocytes (DLI) in HSCT patients with PTLD. A more recent study reported complete or partial remissions in 73%, although GVHD complicated longer-term outcomes in 17% [265]. The use of polyclonal, EBV-specific CTLs prepared from donor leukocytes (reviewed in Refs. [5, 223] yielded response rates (CR and partial remissions (PR)) of 68 and 84% in two recent HSCT series [224, 265]; no patient developed GVHD. Responders experienced exponential increase in the EBV-specific CTL population within 7–14 days with subsequent clearance of EBV viremia [265]. While more extensive disease was associated with treatment failure, four of six patients with CNS involvement achieved CR. Both DLI and EBV-CTL were able to rescue patients failing rituximab therapy, although the response rate was not as high as when adoptive immunotherapy was used initially. Limited data are available concerning the efficacy of adoptive transfer of autologous EBV-specific CTLs in SOT, although preliminary data suggest that regression of disease may occur (reviewed in Refs. [223, 225, 228]. The utility of cloned third-party EBV-specific CTLs was demonstrated in a phase II clinical trial involving 33 patients (2 HSCT, 31 SOT) who had failed conventional therapy with immunosuppression reduction ($N=33$), rituximab ($N=12$), chemotherapy, and/or radiotherapy ($N=8$) [266]. Administration of third-party EBV-specific CTL selected by best available HLA match was associated with a 52% response rate at 6 months with 14 patients achieving a CR; responses were superior with better HLA-matching between the recipient and the CTL donor. In a study of HSCT recipients with PTLD resistant to conventional therapy, responses were achieved in 4 (3 CR, 1 PR) of 11 patients [267]. Recent technologic advances that have lowered cost and decreased the complexity and time for EBV-CTL production should make adoptive immunotherapy more clinically accessible. At present, it has not been recommended in SOT outside of a clinical trial [249]. If adoptive immunotherapy is to be considered in the setting of SOT, it would be critical to determine whether the PTLD lesions are of donor or recipient origin.

26.10.3 Antiviral Drugs and Immunoglobulin

Although EBV lytic transcripts are expressed in some cells in most EBV-positive PTLD [268, 269], the role of antiviral drugs or immunoglobulin as a therapeutic agent in patients with established PTLD is uncertain. At best, these agents may reduce the recruitment of new B cells into the lymphoproliferative process or may influence the expression of lytic viral proteins that modulate the immune response. Alternatively, they may have an indirect benefit on PTLD development by eliminating other viral infections that act as cofactors in PTLD development. It is likely that that these agents exert greater benefit when they are used either as prophylaxis or preemptively rather than as therapy for established disease.

26.10.4 Interferon-α (Alpha) and Blockade of Interleukin-6

IFN-α (alpha) administration and IL-6 blockade by monoclonal antibodies have been used historically, but both of these therapeutic modalities have limited evidence supporting their efficacy in the treatment of PTLD [261, 270] and generally are not used in current practice.

26.10.5 B-Cell Antibodies

The introduction of rituximab has improved response rates in virtually all B-cell CD20-positive lymphoproliferative disorders when combined with chemotherapy [271, 272]. Several retrospective reviews and phase II clinical trials have confirmed the efficacy of rituximab monotherapy in CD20-positive PTLD post-SOT in patients that fail to respond to reduced immunosuppression. Phase II trials show overall response rates (ORR) of 44–71% and CR rates of 26–53% after 4 weekly doses [273–276]. In the setting of HSCT, no prospective trials have been published, but retrospective series report ORRs of 70–84% [96, 157]. Rituximab is well tolerated with no mortality events directly related to treatment toxicity reported in these trials.

Unfortunately, remissions achieved using rituximab monotherapy are durable in only a subset of patients. In a combined analysis of two prospective phase II trials, 58% of responders progressed within 1 year, and 50% of enrolled patients required further treatment within 6 months of rituximab monotherapy. Patients with one or more risk factors, including age ≥60, ECOG performance status ≥2, or elevated LDH, were more likely to relapse and required further treatment [277]. Repeating a course of 4 weekly rituximab doses in patients that initially achieved PR did improve response rates, but did not eliminate subsequent progression [275]. Patients that progress after rituximab remain salvageable with chemotherapy [278].

In summary, while PTLD responds to single-agent rituximab in most patients with very few adverse effects, responses tend to be durable in a minority of patients, necessitating further definitive treatment.

26.10.6 Cytotoxic Chemotherapy

SOT patients with CD20-positive PTLD that fail reduced immunosuppression and rituximab may benefit from chemotherapy. The standard of care in non-transplant aggressive B-cell NHL, and therefore the default regimen in PTLD, is currently rituximab combined with CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) [148, 271, 272]. Historically, chemotherapy has been associated with high treatment-related mortality (TRM) in SOT patients, largely from infectious complications. The improvement in TRM seen in recent studies may be due to improved supportive care measures. However, cytotoxic chemotherapy is often not feasible in HSCT patients due to bone marrow toxicity, and (limited) published response rates are unfavorable [259].

Accurate assessment of remission rates of PTLD in SOT treated with chemotherapy and identification of a superior chemotherapy regimen are elusive, due to the almost entirely retrospective nature of the publications. Results of retrospective studies of other anthracycline-based chemotherapy, mainly CHOP with or without rituximab, show ORRs of 65–73%, 5-year OS of 25–78%, and TRM of 0–31% [279–283]. In one of the few prospective studies, investigators treated 16 adult patients with EBV-related PTLD using a sequential protocol starting with reduced immunosuppression, followed by interferon-α (alpha) and then ProMACE-CytoBOM for those not achieving CR at any step. ORR to reduced immunosuppression, IFN-α (alpha), and chemotherapy were 12.5, 7, and 67%, respectively, and the median OS was only 19 months [261]. A low-dose chemotherapy protocol (cyclophosphamide and prednisone) has been studied prospectively in children post-SOT with EBV-positive PTLD that failed reduced immunosuppression; CR rate was 84%, 2-year OS and event-free survival (EFS) were 73 and 67%, respectively, and TRM was 6% [284]. A subsequent study in a larger cohort added 3 weekly doses of rituximab in the first 2 cycles but saw a lower CR rate (69%) and similar 2-year OS (83%), EFS (71%), and TRM (5%) [285]. The heterogeneity of transplant and histologic subtypes between the studies precludes comparisons to determine the added benefit of rituximab.

The PTLD-1 trial, the largest phase II international multicenter trial in adult PTLD post-SOT, utilized a sequential treatment approach in an attempt to improve the suboptimal relapse rate with rituximab and diminish the toxicity of chemotherapy [258]. Seventy patients with CD20-positive PTLD (mainly late-onset and monomorphic) that failed reduced immunosuppression but had a good performance status were given 4 weekly doses of rituximab followed by four cycles of CHOP every 21 days. Support with granulocyte-colony stimulating factor was required and prophylactic antibiotics were recommended. They reported an ORR of 60% after initial rituximab monotherapy, increasing to 90%, the highest ever reported in any PTLD trial, after sequential chemotherapy. EBV-positive and -negative PTLDs responded equally. Seventy-four percent (74 %) of responders remained in remission 5 years later. There were no TRM events related to rituximab, and TRM related to CHOP was 11%; serious infections related to chemotherapy were more frequent in rituximab non-responders (58 vs. 30%, $p=0.025$). Sequential therapy produced a longer median OS (6.6 years) than rituximab monotherapy trials (1.2–3.5 years) [273–275].

An important question not yet addressed by a prospective trial is whether patients with an excellent response to rituximab alone could be managed without additional chemotherapy. Trappe et al. analyzed prognostic factors in PTLD-1 patients and predicted that patients in CR after four doses rituximab and those in PR with a low-risk international prognostic index (IPI) score were unlikely to have derived benefit from chemotherapy [286]. A prospective phase II multicenter clinical trial to examine risk-stratified sequential therapy (RSST) is currently underway; those who achieve CR with rituximab do not proceed to RCHOP [287]. Preliminary data are promising for 91 patients treated with RSST: 3 year OS was higher with RSST (70%) than with sequential therapy in PTLD-1 (61%). Twenty-seven percent achieved CR with rituximab alone, and only three of these patients have relapsed. The publication of the experience of the PTLD Study Group with RSST is highly anticipated, because it may inform preselection of those likely to avoid chemotherapy without sacrificing disease control.

In summary, in the absence of prospective clinical trials comparing rituximab monotherapy, sequential rituximab followed by CHOP (±R), and RCHOP for the treatment of CD20-positive PTLD in SOT, the standard of care remains elusive. Prior to the PTLD-1 trial publication, British experts had recommended rituximab alone for clinically low-risk PTLD, defined as having no risk factors (age >60, elevated LDH, and poor performance status), whereas they had recommended RCHOP for patients with any of the aforementioned risk factors or for patients who fail rituximab monotherapy [249]. Sequential therapy may offer the highest response and survival rates published to date for CD20-positive PTLDs that fail reduced immunosuppression. While the avoidance of chemotherapy in low risk patients is desirable, patient selection remains unclear [287].

Rare subtypes of PTLD that resemble non-transplant lymphomas, such as Burkitt lymphoma, T cell lymphomas, and plasmablastic PTLD, require specific chemotherapeutic treatment extrapolated from the lymphoma literature (reviewed in Refs. [148, 249]). Treatment for primary CNS PTLD is discussed in a separate section.

26.10.7 Surgical Resection or Radiation

Patients with localized PTLD, such as isolated skin, GI, or renal allograft lesions, can achieve prolonged remissions with surgery or localized radiation [146, 260]. Surgical

resection can be used for tumor debulking, as in allograft PTLD, or management of local complications, such as gastrointestinal hemorrhage, obstruction or perforation in cases of GI PTLD. In cases of PTLD with GI involvement, excess early mortality often results from excellent systemic treatment response causing GI perforation, and some experts consider surgical resection prior to initiating systemic therapy if GI disease is localized [148].

Radiation may be used for patients with life-threatening obstructive or compressive symptoms, and may also be useful for palliation in those with obstructive symptoms that fail to respond to systemic therapy [249]. However, radiation is not considered curative in most cases, and patients with monomorphic PTLD should be treated primarily with systemic therapy [249]. Radiation is potentially curative for plasmacytoma-like PTLD [177].

Radioimmunotherapy using ^{90}Y-Ibritumomab Tiuxetan, or Zevalin, a CD20-directed radiotherapeutic antibody, has produced prolonged remissions in 8 PTLD patients who had failed reduced immunosuppression and rituximab with or without chemotherapy [293]. ORR was 62.5%, and at a median follow-up of 37 months, four patients remained in CR. Toxicity was primarily hematological, and no TRM or graft rejection occurred. Therefore, Zevalin may be an effective salvage treatment for relapsed or refractory PTLD; however, cost and accessibility outside clinical trials may limit its use.

26.10.8 Treatment of PTLD Affecting the Central Nervous System

CNS involvement by PTLD portends a poor prognosis [100, 154, 196]. Management of lymphoma in the CNS involves a different approach than systemic PTLD due to the poor CNS penetration of standard agents, including rituximab. Favored regimens in non-transplant primary CNS lymphoma include high-dose methotrexate (HD-MTX) (3.5–8 g/m^2) alone or combined with cytarabine followed by consolidation of remission using autologous HSCT or whole brain radiation (WBRT) [197]. However, the toxicity of these regimens precludes many patients with SOT and HSCT, as MTX is renally excreted and should be avoided in those with renal or hepatic impairment. Several case series have reported success with HD-MTX in highly selected patients with primary CNS PTLD with reasonable tolerability [288, 289]. In the largest reported retrospective series of primary CNS PTLD ($n=84$), patients treated with rituximab and/or cytarabine (most often given after MTX) tended to have better outcomes, although the optimal treatment regimen was not discernible due to the significant variation in approaches [155]. Overall, lack of response to initial therapy was the strongest predictor of adverse survival (HR 8.7). The retrospective nature of these studies precludes definitive conclusions, but it appears that young patients with primary CNS PTLD should be offered MTX and rituximab if performance status and organ function allow [148, 249]. There are several reported cases of CNS PTLD, particularly early posttransplant, responding to reduced immunosuppression and rituximab monotherapy [155, 290, 291]; which patients would be suitable for this conservative approach is unclear.

Radiotherapy may be useful in the setting of CNS disease, particularly in patients ineligible for chemotherapy due to renal impairment and/or poor performance status and in those that progress or have residual disease following chemotherapy [148, 197]. Although CNS lymphoma is radiosensitive, CRs are rare and the vast majority relapse [197]. WBRT may provide improved disease control in patients with partial remission after chemotherapy [292]. Unfortunately, brain radiation is known to cause significant CNS side effects, such as memory loss [197].

26.10.9 Prognosis with Treatment

Despite advances in prevention, diagnosis, and therapy, mortality from PTLD remains excessive. Lymphomas were the most common cause of cancer-related mortality in SOT patients in a recent study of Australian thoracic and liver transplant recipients, and the risk of death from NHL was 17-fold higher than the general population [294]. In another study, however, a comparison of mortality from lymphoma subtypes in SOT to outcomes of non-transplant lymphomas in a registry yielded no significant difference [147], suggesting that the excess mortality from NHL in SOT patients reflects the increased incidence [294]. Patients with PTLD have a five-fold higher rate of graft failure [295]. Retrospective series of PTLD post-SOT report OS of 30–68% at 5 years, with excess mortality in the first year post-diagnosis [100, 110, 115, 154, 196]. Treatment with rituximab did improve survival in a recent retrospective series [100]. A large UK series of PTLD after HSCT reported 1- and 2-year OS of 46 and 40%, respectively [157].

Several groups have published retrospective studies that identify clinical prognostic factors, but their applicability is limited by small patient numbers, heterogeneous histologic subtypes, and treatment modalities. Previously identified adverse prognostic factors include monomorphic histology, T-cell histology, lack of allograft involvement, bone marrow involvement, CNS involvement, advanced stage, poor performance status, elevated LDH, and hypoalbuminemia [99, 100, 116, 147, 196, 274]. The IPI, a clinical score validated in aggressive NHL [296], was predictive of outcome in some PTLD series [99, 147, 286]. A prognostic score was developed from 500 PTLD cases in renal transplant patients incorporating elevated LDH, disseminated disease, monomorphic disease, serum creatinine >133 μmol/L, and age >55 years; predicted 10-year OS was 85% for low-, 80% for moderate-, 56% for high- and 0% for very high-risk scores [154]. Independent predictors of adverse OS in patients treated in

the PTLD-1 prospective trial were advanced age (>60 years), thoracic transplant, and poor response to rituximab monotherapy [286]. In contrast to earlier studies [153, 297], neither late PTLD nor EBV-negativity was predictive of outcome in several more recent series [84, 100, 154, 277, 286, 298].

Adverse prognostic factors for OS of recipients of T-cell depleted HSCT with PTLD treated with rituximab were age >60, advanced stage, over 1 extranodal site, poor performance status, and absence of peripheral lymphadenopathy. Furthermore, rituximab failure resulted in extremely poor prognoses [157]. Other adverse prognostic factors reported for PTLD in HSCT treated with rituximab include age ≥30, acute GVHD, and no reduction in immunosuppression at PTLD diagnosis [96].

26.10.10 Retransplantation After Posttransplant Lymphoproliferative Disorder

Successful retransplantation in kidney, liver, and cardiac allograft recipients who have recovered from PTLD has been reported. In an analysis of the UNOS database, 69 patients (27 kidney, 22 liver, 9 lung, 6 heart, 4 intestine, 1 pancreas) were identified who underwent repeat transplantation after an episode of PTLD [299]. One third of patients had developed PTLD within the first posttransplant year with only 1.4% diagnosed beyond the first decade. The interval between PTLD and retransplantation was greater than 1 year in 75%. The majority were children at the time of first transplant, suggesting that many may have developed PTLD in the setting of a primary EBV infection. No recurrent PTLD was identified in this cohort, suggesting that retransplantation is appropriate as long as patients have remained disease-free for a significant interval and an immune response to the initial EBV infection has occurred. The optimal time from PTLD remission to retransplantation is unknown, and in the setting of liver, heart, and lung transplantation, timing is largely dictated by clinical need. At present, no data suggest that any one immunosuppressive protocol is superior to another when retransplantation is performed in patients with prior PTLD. In general, there is a trend to avoid induction with anti-thymocyte preparations.

26.10.11 Viral Load Testing to Monitor Treatment Response and Predict Relapse

In some SOT and HSCT recipients with EBV-positive PTLD and high VL at diagnosis, treatment is associated with a fall and clearance of VL coincident with clinical and histologic regression [204, 300, 301]. In a multicenter study of HSCT recipients with PTLD treated with rituximab, Styczynski et al. [96] observed that an increase in EBV DNAemia 1–2 weeks after treatment was a predictor of poor response and was associated with an increased risk of death. However, virologic and clinical responses are not always concordant in SOT PTLD patients receiving rituximab [302, 303] or adoptive immunotherapy with EBV-specific CTL [223]. In SOT pediatric patients, particularly those experiencing primary infection after transplant, asymptomatic intermittent or persistent VL rebound occurs frequently with no short-term consequences [300, 301]. These data, which involved VL monitoring in cellular blood compartments, suggest VL monitoring to measure PTLD therapy response or predict relapse in the SOT setting cannot be recommended. However, as noted earlier (see Sect. 26.8) plasma may be the preferred sample type in this setting [52, 204].

26.11 Potential Future Options for Treatment and Prevention of PTLD

The presence of the EBV genome in all EBV-PTLD cells represents an opportunity for highly "tumor-specific" targeting of therapy. Several investigators have explored the concept that inducing lytic infection in these latently infected cells could cause cytotoxicity and this effect could be further accentuated by the addition of anti-herpesvirus drugs such as acyclovir or ganciclovir [304]. Studies in EBV-positive lymphoid cell lines and animal models suggest that a number of agents used for treatment of hematologic malignancies including doxorubicin, gemcitabine, rituximab/dexamethasone, bortezomib, cyclophosphamide, and γ (gamma)-irradiation induce lytic phase EBV gene expression and the concomitant use of antiviral drugs like ganciclovir increases cytotoxicity [305, 306]. The use of short hairpin RNA to inhibit TERT, a key component of the telomerase complex necessary for EBV-cellular transformation, combined with ganciclovir has demonstrated similar effects as reviewed in Ref. [307].

A number of histone deacetylase inhibitors including butyrate and valproic acid are potent inducers of EBV lytic phase gene expression [304]. In a pilot study, the combination of arginine butyrate and ganciclovir induced complete clinical remissions in four of six patients with PTLD refractory to conventional chemotherapy and/or radiation [308]. In a phase I/II trial using a similar protocol and inclusion criteria, 10 of 15 patients with EBV-associated lymphoid malignancies experienced regression of tumor with four CR and six PR; one third of enrolled patients had PTLD [309]. Two newer histone deacetylase inhibitors suberoylanilide hydroxamic acid and romidepsin have been approved for the treatment of T-cell lymphomas [310]. Determining the impact of this class of drug or chemotherapeutic agents with EBV lytic induction activity when combined with antiviral

drugs on EBV-positive lymphoproliferative disorders requires further clinical trials in both the general population and transplant recipients.

Other EBV-targeted therapies being explored in preclinical studies include the elimination of episomal EBV genomes by low dose hydroxyurea treatment, use of anti-sense RNA against the LMP-1 oncoprotein, expression of detrimental cellular proteins using an EBV-specific promoter dependent expression vector, and the use of small molecules that block signaling pathways constitutively activated by EBV [304, 311]. Manipulation of virally encoded miRNA and virally induced cellular miRNAs is a strategy of significant interest for the development of novel therapeutics [29, 312]. However, targeted delivery of some of these potential therapeutic agents to tumor cells is an unresolved challenge.

A number of new therapeutic approaches are currently being investigated for NHL in the immunocompetent host [313]. Most of these are novel molecularly targeted therapies in various stages of development such as proteasome inhibitors (e.g., bortezomib), immunomodulatory drugs (e.g., lenalidomide), B-cell receptor signaling inhibitors (e.g., ibrutinib), apoptosis effectors (e.g., venetoclax), and novel monoclonal antibodies (e.g., obinutuzumab). Gene expression profiling and next-generation sequencing studies have identified that DLBCL can be further categorized by cell-of-origin (activated B cell (ABC) or germinal center B cell (GCB)), and that these subtypes may be dependent on unique molecular pathways. Monomorphic B cell PTLDs are more often ABC subtype [116]. The targeted novel therapies showing more promise in ABC subtype include ibrutinib and lenalidomide. Whether these agents will be used alone or in combination with chemotherapy has yet to be determined.

Bexarotene, a synthetic retinoid analogue approved for use in cutaneous T-cell lymphoma, may have activity against other forms of T cell-derived PTLD. Bexarotene has been used with good effect in a single case of refractory EBV-associated peripheral T-cell PTLD where it appeared to induce remission [314]. Other novel agents holding promise in the treatment of non-transplant T-cell lymphomas include romidepsin and belinostat (histone deacetylase inhibitors), and brentuximab vedotin (an anti-CD30 monoclonal antibody) [315].

Interest has recently turned to the potential use of pamidronate, a widely available bisphosphonate that reduces bone turnover, in the treatment of PTLD (reviewed in Ref. [316]). It activates and expands a class of λδ (gamma delta) T cells that have NK cell characteristics and participate in the innate immune system. In vitro, these cells led to lysis of EBV-transformed autologous human lymphoblastoid B-cell line. In a murine model of human B-cell lymphoproliferative disease, adoptive transfer of these pamidronate-expanded λδ (gamma delta) T cells both prevented the development of disease when given preemptively and led to remissions and prolonged survival in mice with established disease [317]. Similar results were seen when tested in a murine model where immunodeficient mice had been reconstituted with a functional human immune system. Whether these preliminary results will translate into clinical efficacy has yet to be determined.

Research in vaccine development has focused on strategies that would affect EBV-associated disorders, such as PTLD, Burkitt lymphoma, and nasopharyngeal carcinoma (reviewed in Ref. [318]). A recombinant gp350 vaccine reduced the rate of symptomatic infectious mononucleosis from 10 to 2%, although it failed to reduce the rate of asymptomatic primary infection [319]. Because the theory of PTLD pathogenesis suggests that very high VLs may be important, vaccines that lower VL in infected patients, even if not preventing infection, may reduce PTLD risk. The EBV-seronegative patient awaiting SOT may be a particularly important subgroup in whom to further evaluate this vaccine. A phase I clinical trial of the gp350 vaccine in EBV-seronegative children with chronic kidney disease ($N=16$) failed to prevent either wild-type EBV infection pretransplant or reduction in EBV VLs when compared to non-vaccinated patients undergoing primary EBV infection posttransplant [320]. Neutralizing antibody developed in the minority and titers declined rapidly. Moreover one of the vaccinated children developed PTLD. Whether better adjuvants or a modified vaccination schedule would produce greater vaccine efficacy requires further testing. Immunosuppression due to the presence of uremia may also have had an impact.

An alternative vaccine strategy is aimed at the generation of a CTL response to latently infected cells by using formulations of synthetic peptides that mimic immunodominant epitopes recognized by EBV-induced $CD8^+$ CTLs in vivo. Recently, a phase I trial of such a vaccine has revealed that it is well tolerated and induced EBV-specific CTL in healthy volunteers [321]. The development of this type of vaccine faces the immense obstacle of including sufficient peptides in the formulation to address the large number of HLA polymorphisms in the population.

26.12 Summary

Significant progress has been made both in the understanding of the biology of EBV infection and in the pathogenesis of PTLD. This has resulted in major advances in the prevention and treatment of EBV-associated PTLD occurring early after transplantation. Nonetheless, morbidity and mortality associated with PTLD persists. As transplant recipients survive longer, the risk of late EBV-negative PTLD has become more significant. A standardized approach to pathology is critical for both understanding pathogenesis and designing therapy. New techniques for monitoring EBV VL hold promise, but they suffer from a lack of standardization. Prospective, multicenter, controlled trials evaluating prophylactic,

preemptive, and therapeutic strategies are needed to reduce the incidence, morbidity, and mortality associated with this complication of transplantation.

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27
Herpes Simplex and Varicella-Zoster Virus Infection after Hematopoietic Stem Cell or Solid Organ Transplantation

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27.1 Introduction

Because of the impaired cell-mediated immunity that accompanies antirejection therapy, clinical manifestations of herpesvirus diseases in organ transplant recipients are more frequent, severe, and prolonged than those in immunocompetent individuals [1]. A key characteristic of herpesviruses is establishment of latent infections of specific tissues that persist for life. The viral genome is present within latently infected cells, but does not undergo full replication cycles to produce infectious progeny. Nevertheless, immune-mediated eradication of the infection does not occur. Periodically, latent HSV and VZV will replicate and temporarily overwhelm the immune response, resulting in herpes labialis or herpes zoster.

Infections with herpes simplex virus (HSV) and varicella-zoster virus (VZV) are common (>50 % seroprevalence in most populations). Most primary infections occur during childhood and diagnosis of latent infections is established by serological testing. Diseases in adult transplant patients are usually due to reactivation. When primary infection occurs in transplant patients, manifestations are frequently severe.

While CMV causes more than half of viral infections in transplant patients, HSV and VZV are the next most common viral infections [1–5]. The intensity of immunologic suppression is associated with frequency and severity of disease. For example, the VZV attack rate is five times higher after allogeneic hematopoietic stem cell transplantation (HCT) than renal transplantation. Reduction in immunosuppressive therapy may be necessary to achieve control of life-threatening infections. While prophylactic antiviral therapy reduces the risk of herpesvirus disease, the ultimate goal is to develop selective immunosuppression that prevents graft rejection without suppressing antiviral cellular immune responses.

27.2 Description of the Pathogens

Herpes simplex viruses type 1 and type 2 (HSV-1, HSV-2) and VZV are alpha herpesviruses, characterized by similar structures, rapid replication cycles, destruction of host cells, lifelong latency in sensory nerve ganglia, and a robust cell-mediated immunologic response in tissue. Each virion is 100–200 nm in diameter. The nucleocapsid is composed of 162 capsomeres arranged in icosahedral symmetry. An amorphous tegument layer surrounds the capsid. The virion is enclosed in a lipid-containing laminated envelope with external glycoproteins that mediate entry into cells and serve as antigens. The herpesvirus genome consists of a linear, double-stranded DNA molecule (120–230 kb) with unique regions flanked by terminal repeating sequences. Herpesviruses specify a variety of enzymes to catalyze steps in the replication cycle, including nucleic acid metabolism, DNA synthesis, and protein processing. Synthesis of viral DNA and capsid assembly occurs in the nucleus; the envelope is acquired as the capsid passes through the nuclear membrane. Productive infection with release of progeny virus results in cell death.

27.3 Herpes Simplex Virus

27.3.1 Epidemiology

HSV-1 is transmitted most commonly during childhood by oral secretions. Since HSV-2 is sexually transmitted, seroprevalence remains low until puberty. In the USA, HSV-1 and HSV-2 seroprevalence is 62 % and 16 %, respectively, with significant variation by age and socioeconomic groups [6, 7]. A recent trend in developed countries is that HSV-1 is a more common cause of incident genital herpes than HSV-2, though the long-term recurrence rates of genital HSV-1 may be attenuated relative to genital HSV-2 [8]. In the modern treatment era, the incidence of HSV disease in HCT patients is determined by the duration of antiviral prophylaxis: the 2-year incidence of HSV disease in patients receiving 30 days of prophylaxis was 32 % versus 0.2 % in patients receiving a full year of prophylaxis [9].

Clinical manifestations of HSV occur with increased frequency in patients with reduced cell-mediated immune responses due to disease or immunosuppressive therapy.

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Prior to routine antiviral prophylaxis, HSV disease was noted in 70–80 % of HCT and 40–50 % of solid organ transplant (SOT) recipients [10–16]. Cumulative incidence in the era of antiviral prophylaxis is considerably lower.

27.3.2 Pathogenesis

In a newly infected individual, viral replication and spread occurs within keratinocytes on the mucocutaneous surface, followed by viral transport via cutaneous neurons to nerve cell bodies in ganglia where latency is established. When latent HSV reactivates, virus travels via sensory nerves back to the skin, where replication occurs in epidermal layers, sometimes producing clusters of painful vesicles. For HSV-1 and HSV-2, most shedding is asymptomatic, which is critical for transmission [17].

The immune mechanisms that control HSV reactivation are not fully elucidated. $CD8^+$ T lymphocytes surround latently infected neurons, and persistent $CD8^+$ cytotoxic T-lymphocyte (CTL) activity has been demonstrated in infected patients [18, 19]. HSV-2 specific T-cell aggregates persist at mucosal lesion sites for months. The spatial heterogeneity of these cells may contribute to the episodic nature of -2 shedding over time [20, 21]. Both adaptive and innate responses can be suppressed by antirejection immunosuppressive therapy. Donor anti-HSV immunity is transferred during HCT and provides protection against recurrent disease, dissemination, and development of antiviral drug resistance [22].

Most HSV infections that occur following HCT or SOT result from reactivation of endogenous virus, rather than primary infection. Primary infections account for ~2 % of HSV disease in HCT recipients [23]. Determination of HSV-1 and HSV-2 serologic status prior to transplantation aids subsequent prophylactic strategies. Asymptomatic oropharyngeal shedding is common in seropositive transplant patients not receiving antiviral prophylaxis [24, 25]. A subset of these patients will develop orofacial lesions. Patients occasionally transmit virus to remote anatomic sites via autoinoculation, resulting in syndromes such as herpes keratitis or whitlow.

Herpesviruses can be transmitted via a solid organ graft from a seropositive donor [26–28], though this has not been observed following HCT. If the recipient is seronegative, primary infection occasionally results in viremia, which can lead to lethal, visceral organ involvement. Two patients who received kidneys from the same donor developed fulminant, fatal HSV-2 hepatitis. Isolates from both recipients had identical restriction endonuclease patterns, proving transmission via the transplanted organs [27]. No such cases have been observed in the era of antiviral prophylaxis.

In the absence of prophylaxis, most HSV infections occur within 30 days after SOT or HCT [13, 14, 28–33]. HSV reactivates rapidly after initiation of immunosuppressive therapy, which coincides with suppression of lymphocyte responsiveness to HSV antigens [34, 35]. Intensity of immunosuppression predicts risk of posttransplant HSV disease, with higher rates in SOT patients who received OKT3 or antithymocyte globulin [13, 36], and after haploidentical transplant [37]. No correlation between HSV antibody titers and disease has been established. Donor immunity does not prevent reactivation in the graft recipient, but does limit recurrence and drug resistance. Following SOT, lymphocyte responsiveness to HSV antigens normalize within about 6 months, and recurrence frequency declines [34]. In HCT recipients, reestablishment of specific lymphocyte reactivity is dependent on reexposure to HSV antigens [35]. Patients who develop a lymphocyte response to HSV after first posttransplantation recurrence maintain a lower frequency of subsequent recurrences [35]. In HCT populations, acute graft-versus-host disease (GVHD) [38] or a matched-unrelated/mismatched donor [39] are associated with increased HSV disease risk. No detrimental effect of HSV infection on graft survival has been demonstrated.

27.3.3 Clinical Presentation and Natural History

Seventy to eighty percent of seropositive HCT patients will develop HSV disease [13, 14, 24, 35, 40, 41]. Certain chemotherapies, including lenalidomide for multiple myeloma, are associated with high incidence of HSV disease. These high attack rates provide rationale for antiviral prophylaxis to prevent HSV during the weeks after transplantation.

The initial presentation of mucocutaneous disease in transplant patients often does not differ from infection in immunocompetent hosts. However, a subset of lesions in immunocompromised patients heal more slowly, and a smaller percent undergo fatal dissemination [14, 40, 42].

Herpetic lesions begin as painful erythematous papules that progress to clusters of vesicles. If vesicles remain intact, they evolve into pustules with the influx of inflammatory cells. Vesicles are fragile and rupture results in erosions on an erythematous base that crusts. Healing occurs over 5–10 days in normal hosts, but may take 4–6 weeks in immunocompromised hosts off therapy [40, 42]. In some patients, large, atypical, mucocutaneous ulcerations develop that require biopsy or viral culture for accurate diagnosis.

Orolabial lesions account for most HSV disease in after transplant [23]. Lesions involve the lips, gingiva, tongue, posterior pharynx, and perioral skin. In HCT patients, intraoral HSV disease during the granulocytopenic period is difficult to distinguish from chemotherapy induced mucositis [43]. If typical lesions are present, the diagnosis of HSV gingivostomatitis is suggested but should be confirmed with a viral culture or assay for viral DNA, particularly if there is concern for drug resistance. Herpetic gingivostomatitis can cause severe pain resulting in diminished nutritional intake and possible bacterial superinfection.

Anogenital herpes usually results from HSV-2 reactivation in sacral ganglia [44], and ranges from self-limited lesions to extensive chronic ulcerations that persist for weeks [45, 46]. Cases of atypical HSV that mimic HPV disease, hemorrhoids, or malignancy have been noted in transplant patients [44–47]. Anogenital HSV-2 may be complicated by lymphocytic meningitis, which is usually self-limited and does not progress to encephalitis.

Mucocutaneous HSV infections can cause serious morbidity, but are usually self-limited. HSV dissemination can result in visceral organ infections that are difficult to diagnose and frequently lethal. HSV can spread by direct extension from the oropharynx to the gastrointestinal or respiratory tracts, causing esophagitis or pneumonia. The presence of a nasogastric tube may increase risk of esophagitis in a transplant patient with oral HSV. HSV may mimic skin GVHD with histologic features resembling HSV associated erythema multiforme [48]. HSV encephalitis does not occur with increased frequency in transplant patients. Viremic dissemination to multiple organs, while rare, is associated with high mortality.

Patients with HSV esophagitis present with dysphagia and odynophagia that may be indistinguishable from syndromes caused by *Candida* or CMV [49, 50]. Because clinical and radiographic findings cannot distinguish among the etiologies of esophagitis, endoscopy for biopsy and cultures is required. Endoscopic examination reveals herpetic superficial erosions and ulcerations that become confluent [49, 50]. Colitis in transplant patients is much more commonly caused by CMV than by HSV in the era of antiviral prophylaxis [51, 52].

HSV pneumonia is more common in HCT, lung, and heart–lung transplant recipients, than other SOT patients [12, 53–55]. Pulmonary HSV involvement with HSV most commonly results from aspiration or local extension from the upper airway, rather than viremia [56]. HSV-1 pneumonia resulting from contiguous spread is characterized by focal or multifocal infiltrates on chest radiograph. Endotracheal intubation increases the risk of direct extension to the tracheobronchial tree. HSV pneumonia resulting from viremia may be due to either HSV-1 or HSV-2, presents with diffuse infiltrates, and is usually accompanied by disease affecting other organs [57]. HSV pneumonia presents with fever, cough, dyspnea, hypoxemia, and chest radiograph abnormalities that may be indistinguishable from bacterial pneumonia [54]. Chest computed tomography may demonstrate focal consolidation with small centrilobar nodules and areas of ground-glass attenuation [56]. Bronchoscopy reveals tracheobronchitis. Since HSV is frequently present in oral secretions, positive cultures from expectorated sputum are misleading. A definitive diagnosis of HSV pneumonia is based on positive viral culture or polymerase chain reaction (PCR) of deep lung specimens, coupled with histopathologic evidence of infection on biopsy.

Viremic HSV-1 or HSV-2 dissemination can result in infection of the liver, lungs, adrenal glands, gastrointestinal tract, and skin during the first 30 days after transplant. This syndrome has been described most frequently in renal and liver transplant patients, but also in HCT recipients [26, 27, 58–62]. Systemic HSV usually occurs within 1 month of transplantation. Primary HSV infection with dissemination has occurred when an organ is transplanted from a seropositive donor to a seronegative recipient [26, 27, 63], though reactivation in seropositive patients is the more common etiology [64]. Computed tomography may demonstrate innumerable tiny low-density hepatic foci that may be mistaken for *Candida* micro-abscesses [28, 65]. Clinical findings included abdominal pain and tenderness, fever, and elevated hepatocellular enzymes. Not all patients have HSV skin lesions. Liver biopsy (for HSV cultures, PCR, and histopathology) [28, 66], or PCR of blood are diagnostic studies of choice, the diagnosis is often made postmortem.

The mortality rate for transplant patients with untreated HSV hepatitis is 60–80 % and approaches 100 % if disseminated intravascular coagulopathy develops [28]. Rapid diagnosis and early antiviral therapy are essential for survival [67], although a few cases of less fulminant hepatitis (>60 days after transplantation) have been reported [68]. Empiric therapy with intravenous acyclovir should be initiated as soon as the diagnosis is considered. Liver transplantation has been attempted in a few patients with liver failure due to fulminant HSV [62, 69–71].

CMV hepatitis is also rare, but presents differently. HSV hepatitis tends to occur within 5–25 days of transplantation is rapidly progressive, and is associated with leukopenia with an increased percentage of band forms [62]. CMV hepatitis usually occurs 20–50 days after SOT, has a subacute course, and is associated with leukopenia and atypical lymphocytes. A liver biopsy is essential to provide an accurate diagnosis, especially in liver transplant recipients in whom the differential diagnosis also includes hepatitis B, hepatitis C, or graft rejection.

Another scenario in which implantation of allogeneic tissue can transmit HSV is ocular surgery, especially keratoplasty or corneal transplantation [72, 73]. HSV transmitted by a donor cornea can result in graft failure and ulcerative keratitis after transplantation [74].

27.3.4 Diagnosis

Typical mucocutaneous herpetic lesions can be accurately identified by experienced clinicians. Atypical lesions or visceral organ involvement requires laboratory confirmation. Intraoral HSV lesions can be difficult to distinguish from mucositis. When performed early during infection, viral culture of lesions is a sensitive technique: vesicle or ulcer exudate is collected on a swab and placed in appropriate transport media. Biopsy specimens can be minced and extracted for culture and should be inoculated onto cells as soon as possible. Specimens can be stored at 4 °C for up to 48 h, although

some reduction in yield may result. HSV grows readily on many mammalian cell lines. Characteristic cytopathic changes can be observed within 24–48 h, though growth occasionally takes a week. Viral typing can be performed by staining cells from the culture monolayer with fluorescein-labeled HSV-1 and 2-specific monoclonal antibodies.

A variety of rapid diagnostic assays to detect HSV antigens or nucleic acids have replaced virus culture at many centers. PCR is the method of choice for diagnosing central nervous system infections, is sensitive for detecting HSV DNA in tissues and blood, and should be employed for culture negative lesions and suspected visceral disease [47, 75–78].

A sensitive direct immunofluorescence assay (DFA) is available for diagnosis of mucocutaneous lesions in under an hour [79]: cells scraped from a vesicle or ulcer base are stained with fluorescein-labeled monoclonal antibodies specific for HSV-1, HSV-2, or VZV.

Because most HSV infections are due to reactivation in patients with preexisting antibody, serology is helpful to define susceptibility, but not to diagnose active disease. A negative assay for anti-HSV antibodies excludes recurrent, but not primary HSV. In the past, most laboratories used a whole-virus enzyme-linked immunosorbent assay (ELISA) that did not reliably distinguish between antibodies against HSV-1 or HSV-2. Current highly specific serologic tests usually allow identification of patients who are infected with HSV-1, HSV-2, or both [80, 81]. In cases of borderline serologic results, or results discordant with clinical history, HSV Western Blot is the gold standard for diagnosis.

27.3.5 Therapy

The release of acyclovir in the early 1980s revolutionized the treatment and prophylaxis of HSV. Small trials of intravenous acyclovir for mucocutaneous HSV infections in immunocompromised patients demonstrated efficacy and safety [82]. Subsequent large, randomized, placebo-controlled trials in BMT and SOT populations with intravenous acyclovir (5 mg/kg or 250 mg/m^2 IV every 8 h) demonstrated reduced duration of shedding (median 2.8 vs. 16.8 days), pain (median 8.9 vs. 13.1 days), and lesions (median 13.7 vs. 20.1 days), compared with placebo [83].

While intravenous acyclovir is preferred for severe, life-threatening infections, oral acyclovir is more convenient for less serious HSV infections, and is safe and well tolerated. In 21 BMT recipients, oral acyclovir reduced the duration of mucocutaneous shedding (median 2 vs. 9 days), pain (median 6 vs. 16 days), and lesions (median 8 vs. 21 days), compared with placebo (Figure 27-1) [84]. Valacyclovir (an acyclovir prodrug) and famciclovir (a penciclovir prodrug) have in vitro antiviral activity similar to acyclovir, but produce higher serum concentrations, which allows simpler dosing schedules. Clinical trials in immunocompetent hosts with valacyclovir and famciclovir demonstrate high effectiveness, though controlled studies with these drugs are limited.

A topical preparation of acyclovir is beneficial for treatment of mucocutaneous HSV in immunocompromised patients [82], but is not as effective as systemically administered drug. There is no role for topical acyclovir or penciclovir in transplant patients given the safety of oral preparations.

No prospective, controlled studies have been performed to evaluate acyclovir in transplant patients with disseminated or visceral HSV infections. However, clinical experience clearly supports the value of high-dose intravenous acyclovir (10 mg/kg IV every 8 h) in this setting [14, 66, 85].

Antiviral therapy of mucocutaneous disease should be continued until lesions are healed, which will usually be at least 10 days. Premature discontinuation can result in relapse and emergence of acyclovir-resistant HSV. Ganciclovir, foscarnet, and cidofovir are primarily used to treat CMV but also have excellent activity against HSV. Acyclovir has

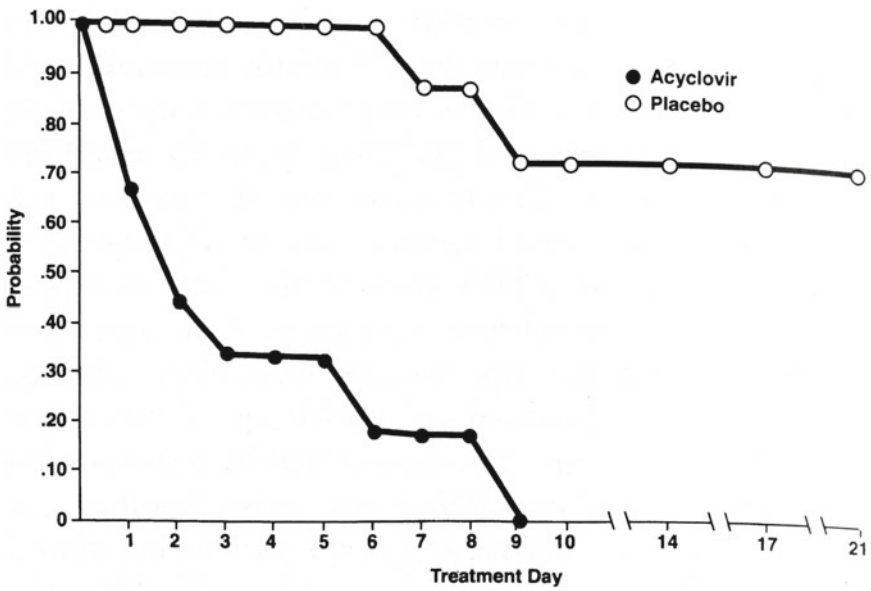

FIGURE 27-1. Time-to-event curves showing probability of remaining culture-positive for HSV, from a study of BMT patients with mucocutaneous HSV disease treated with oral acyclovir (*closed circles*) or placebo (*open circles*) for 10 days ($P = 0.0008$). (Modified from Shepp DH, Newton BA, Dardliker PS, et al. Oral acyclovir therapy for mucocutaneous herpes simplex virus infections in immunocompromised marrow transplant recipients. Ann Intern Med. 1985;102:783–785).

limited therapeutic activity against CMV, though high dose valacyclovir dosed at 2 g every 6 h is effective in preventing CMV reactivation [86]. In transplant patients with concurrent CMV and HSV infections, high-dose ganciclovir, foscarnet, or cidofovir provide effective treatment for both pathogens.

About 2–5 % of HSV isolates recovered from HCT patients are acyclovir resistant; the frequency in SOT patients is lower [87–91]. While HSV incidence following HCT remains low, the percentage of resistant isolates continues to increase with the advent of longer and deeper immunosuppression [92]. The most common manifestation of acyclovir-resistant HSV is extensive, chronic mucocutaneous disease [91]. Serious visceral infections have been reported [88, 93, 94]. Immunocompromised patients with cutaneous lesions or mucositis that fail to heal despite acyclovir therapy should be recultured and submitted for antiviral susceptibility testing [94, 95].

Acyclovir prophylaxis suppresses viral replication and reduces the probability of resistance mutations [95, 96]. In HCT patients, resistance develops more frequently in patients receiving short-term prophylaxis or repeated treatment for recurrent disease than in patients on long-term high-dose (acyclovir 800 mg twice daily or valacyclovir 500 mg twice daily) prophylaxis [9, 24, 91, 96].

The initial phosphorylation step in acyclovir activation is catalyzed by virally encoded thymidine kinase. HSV with mutations that alter thymidine kinase will be resistant to acyclovir [86, 94, 97–99]. Famciclovir and ganciclovir are also dependent on thymidine kinase for activation, and are against most acyclovir-resistant HSV strains. Foscarnet, which is not dependent on thymidine kinase, can be used to treat acyclovir-resistant HSV [88, 100–102]. Foscarnet is more toxic than acyclovir and can lead to renal insufficiency in 25 % of patients as well as electrolyte wasting and aphthous genital ulcers [103]. Infections caused by rare strains resistant to acyclovir and foscarnet may respond to cidofovir [89, 104–106]. Cidofovir is associated with dose-dependent renal insufficiency and a Fanconi like syndrome with proteinuria, glucosuria, and bicarbonate wasting [107]. Cidofovir is co-dosed with probenecid, which is associated with nausea and potentially dehydration. Patients on foscarnet and cidofovir should have their renal function tested frequently and receive vigorous hydration. Brincidofovir (CMX001), an investigational oral prodrug of cidofovir, has greater potency, less toxicity and broad activity against CMV, HSV, adenovirus, smallpox and Ebola virus [108, 109].

27.3.6 Prophylaxis

The widespread adoption of antiviral prophylaxis has dramatically altered the natural history of HSV infections in HCT [106, 110–112]. Prophylaxis minimizes HSV-associated morbidity and mortality in the posttransplant period and reduces emergence of acyclovir-resistant strains [9, 95, 110]. Clinical trials using intravenous acyclovir prophylaxis in BMT patients documented reduction in HSV incidence from 70 % to <5 % [111–113]. Prophylaxis using oral acyclovir was nearly as effective as (Figure 27-2), although some patients in the immediate posttransplant period had difficulty taking pills due to stomatitis or nausea [113–116]. Due to an increased frequency of HSV disease shortly after acyclovir prophylaxis was discontinued, a current highly effective strategy is intravenous acyclovir during the immediate posttransplant period followed by long-term

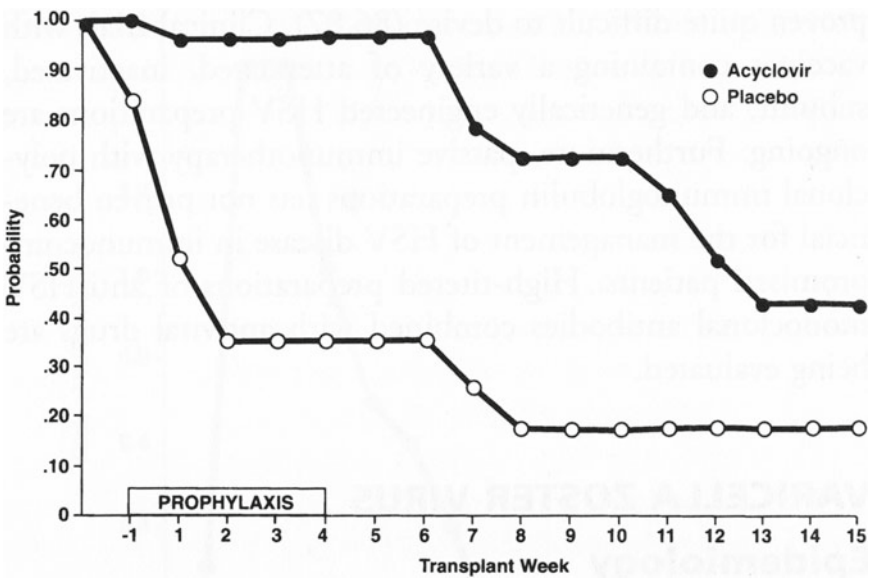

FIGURE 27-2. Time-to-event curves showing probability of remaining culture-negative for HSV, from a study of BMT patients receiving prophylaxis with acyclovir (*closed circles*) or placebo (*open circles*) for 5 weeks ($P=0.0002$). (Modified from Wade JC, Newton B, Flournoy N, et al. Oral acyclovir for prevention of herpes simplex virus reactivation after marrow transplantation. Ann Intern Med. 1984;100:823–828).

oral prophylaxis [112, 115, 117]. An oral acyclovir regimen of 800 mg twice daily has >90 % virologic efficacy and is not associated with development of resistance. This dose is well-tolerated, associated with high patient compliance, and affordable [118–120], and does not delay marrow engraftment [112, 114, 115, 119]. Many centers use acyclovir 400 mg twice daily [121], though this dose has not been tested as extensively for its ability to prevent resistance. Oral valacyclovir is well tolerated in HCT patients, offers a simplified prophylactic dosing regimen [113, 122–124], and is more cost-effective than intravenous acyclovir [113, 123].

acyclovir prophylaxis is also safe and effective in SOT [118, 124, 125], including renal [30, 126], liver [127], and cardiac transplant patients [128, 129]. Ganciclovir or valganciclovir administered for CMV prophylaxis provide effective prevention of HSV disease [106]. Physicians who use a preemptive therapy approach to CMV should use HSV prophylaxis with acyclovir or valacyclovir in the early posttransplant period.

27.3.7 Immunoprophylaxis of HSV Infections

Vaccinating HSV-seronegative transplant candidates is theoretically attractive, but an effective vaccine is not licensed [130]. Clinical trials are ongoing with a variety of attenuated, inactivated, subunit, and genetically engineered HSV preparations. Passive immunotherapy with polyclonal immunoglobulins has not proven beneficial for management of HSV disease in immunocompromised patients.

27.4 Varicella-Zoster Virus

27.4.1 Epidemiology

Humans are the only known reservoir for VZV. In temperate regions, varicella (chickenpox) epidemics occur annually in the late winter and early spring. Prior to introduction of the varicella vaccine in 1995, about 3.8 million annual varicella cases occurred in the USA. About 60 % occurred in children aged 5–9, and 90 % occurred in patients under 15 years of age [131]. Greater than 90 % of the population had varicella before age 20. With widespread vaccination, the chickenpox incidence has dramatically declined [132]. Due to either past infection or vaccination, fewer than 5 % of adult organ transplant recipients in the USA are VZV-seronegative [133].

Herpes zoster (shingles) results from reactivation of latent VZV. The incidence of zoster in the USA is about four cases per 1000 population per year [134–136]. Incidence is age-related, exceeding ten cases per 1000 patient-years in individuals more than 80 years of age [134, 135]. Impaired cell-mediated immunity due to lymphoproliferative malignancies, AIDS, or organ transplantation increases risk for development of zoster [137, 138].

27.4.2 Pathogenesis

Primary infection occurs when a susceptible individual is exposed to airborne VZV. Patients with chickenpox are infectious for 48 h prior to and 4–5 days after rash onset. Varicella is most often acquired from exposure to another individual with chickenpox, but can occur from exposure to a patient with zoster. Rare cases of VZV transmission via the donor organ have been reported when the donor had recent chickenpox [139]. Varicella is highly infectious, with attack rates exceeding 70 % following household exposure.

As VZV replicates in the skin during acute varicella, some virions are transported via sensory nerves to the corresponding dorsal root ganglia, where latency is established. The specific immune responses that limit VZV reactivation from ganglia are incompletely understood, but the most important factor predisposing to the development of zoster is declining VZV-specific cellular immunity, resulting from aging or immunosuppressive illness or therapy [140]. Impairment of VZV-specific effector memory T-cells occurs in SOT patients [141]. Although zoster is usually a disease of adults, VZV-seropositive children who undergo organ transplantation are at high risk for zoster [142]. Following VZV reactivation and replication in ganglion, virus travels along the sensory nerve to the skin, where it replicates in epithelial cells, producing the characteristic painful dermatomal vesicular eruption of herpes zoster (Figure 27-3).

27.4.3 Incidence and Risk Factors

The frequency of zoster in transplant populations correlates with intensity of immune suppression [138]. For HCT, the cumulative risk of zoster at 5 years posttransplant is 15–25 % for autologous and 20–45 % for allogeneic recipients, with most infections occurring within the first year [143–151]. Among SOT recipients, herpes zoster incidence is about

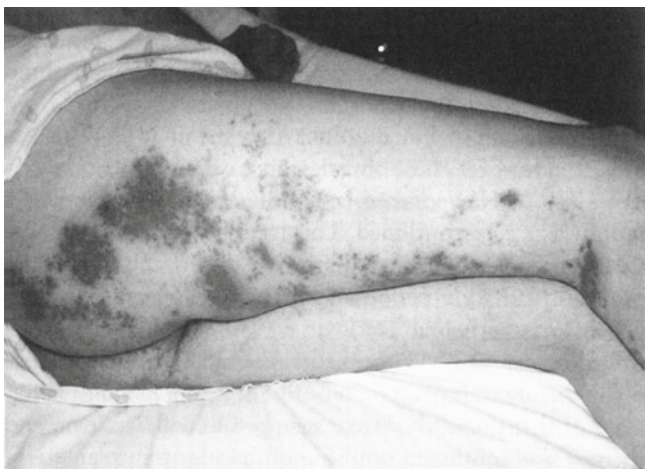

FIGURE 27-3. Herpes zoster in a heart transplant patient involving the right S2 dermatome. Photograph courtesy of John Gnann, MD.

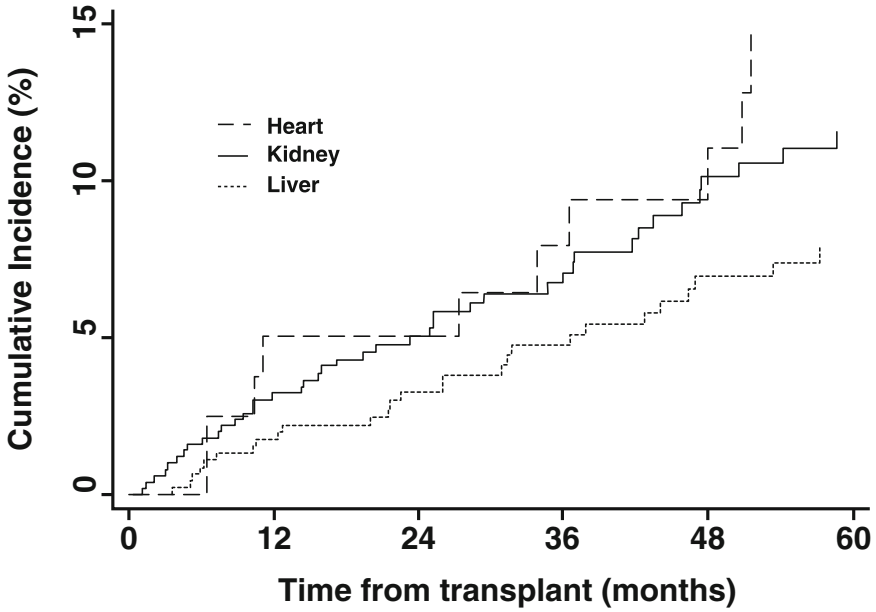

FIGURE 27-4. Cumulative incidence of herpes zoster posttransplant in cohort of VA SOT recipients from 1996 to 2007, by organ type. A log-rank test between curves shows a significant difference between the three groups ($P = 0.007$). (Modified from Pergam SA, Forsberg CW, Boeckh MJ, et al. Herpes zoster incidence in a multicenter cohort of solid organ transplant recipients. Transplant infectious disease: An official journal of the Transplantation Society 2011;13:15–23).

20 cases per 1000 patient-years, although risk varies significantly by organ type. The cumulative 5-year risk for herpes zoster is higher in lung or heart (20–25 %) than in kidney or liver recipients (15–20 %) [133, 152–159] (Figure 27-4). In addition to morbidity and mortality, this high incidence in transplant patients results in substantial resource utilization and cost burden [160].

Several factors predispose transplant recipients to zoster. Among SOT recipients, African-American race and older age are associated with increased relative hazard [156]. Among BMT patients, underlying disease, lymphopenia and GVHD are associated with higher risk. The risk is 46 % among patients transplanted for Hodgkin disease or lymphoma, 23 % for leukemia, and 9 % for solid tumors [151, 161]. Patients with a $CD4^+$ lymphocyte <200 cells/μL and $CD8^+$ lymphocytes <800 cells/μL on posttransplant day 30 had an actuarial risk of 48 % at 1 year [146]. In studies of VZV-seropositive children, the risk of herpes zoster was higher after cord blood than after BMT [158] and higher for allogeneic than autologous transplant [142]. Among allogeneic HCT patients, GVHD probably increases the incidence of herpes zoster and dissemination once zoster occurs [145, 147, 157].

No correlation exists between anti-VZV IgG titers and subsequent risk of herpes zoster [162]. Instead, risk correlates with the level of VZV-specific cell-mediated immunity, which is suppressed after transplant. Higher zoster rates may occur in patients receiving more intensive immunosuppressive therapy, including mycophenolate mofetil or high doses of corticosteroids [155, 159, 163–166]. Bortezomib therapy prior to autologous HCT for multiple myeloma has been associated with increased risk of herpes zoster [167–171].

Following allogeneic HCT, VZV-specific immune responses may be lost, even if the donor and the recipient were seropositive. As with HSV, T-cell recognition of VZV proteins may not be fully reconstituted until the patient experiences reexposure to VZV via clinical disease or possibly subclinical reactivation [172]. However, most patients develop durable immunity, and few experience more than one episode of zoster.

27.4.4 Clinical Presentation and Natural History

27.4.4.1 Varicella

Because most adults are latently infected with VZV, true primary infection occurs mostly in unvaccinated children [173]. Varicella is only occasionally diagnosed in adult transplant patients [166, 174–176]. Rare cases of primary varicella occur in VZV-seropositive HCT patients, indicating reinfection [177]. Determination of VZV serologic status prior to transplantation aids clinical decision-making and prevention strategies. Chickenpox in patients undergoing HCT or SOT has been associated with high rates of morbidity and mortality [166, 176, 178]. Skin lesions may be unusually numerous and severe, with ongoing new lesion formation for up to a week. Cutaneous complications in immunocompromised children include bullous or hemorrhagic lesions, necrotizing fasciitis, purpura fulminans, and bacterial superinfection. Prior to antiviral therapy, visceral involvement (especially

pneumonitis and hepatitis) was reported in nearly half of immunocompromised children with chickenpox, resulting in 10–20 % mortality [179]. VZV pneumonia occurred in 25–30 % of these children, presenting with fever, cough, and dyspnea within 3–5 days after onset of skin lesions. VZV meningitis has also been described in pediatric HCT patients [180]. Potent antirejection therapy and absolute lymphopenia (<500/mm^3) are risk factors for dissemination and death [181]. The availability of effective antiviral therapy has reduced mortality rate to <5 % in transplant patients with chickenpox [182–185].

27.4.4.2 Herpes Zoster

Unlike HSV infections that occur in the immediate post-transplant period, VZV reactivation usually occurs later in transplant patients (Figure 27-5). In the pre-antiviral era, median time to zoster following HCT was 5 months, with >75 % of cases occurring in the first year [161, 186–188]. Widespread adoption of prophylactic antiviral regimens has resulted in a delay in zoster until a median of 7 months post-transplant, when prophylaxis has been discontinued [145]. Only 12 % of the episodes occurred within the first 100 days. Among patients who survived 24 months after transplantation, 59 % developed zoster. In a cohort of 239 lung transplant patients, zoster incidence was 12.1 % and mean time to first episode was 486 (±265) days. The cumulative incidence of zoster was 5.8 %, 18.1 %, and 20.2 % at 1, 3, and 5 years after lung transplantation, respectively [155].

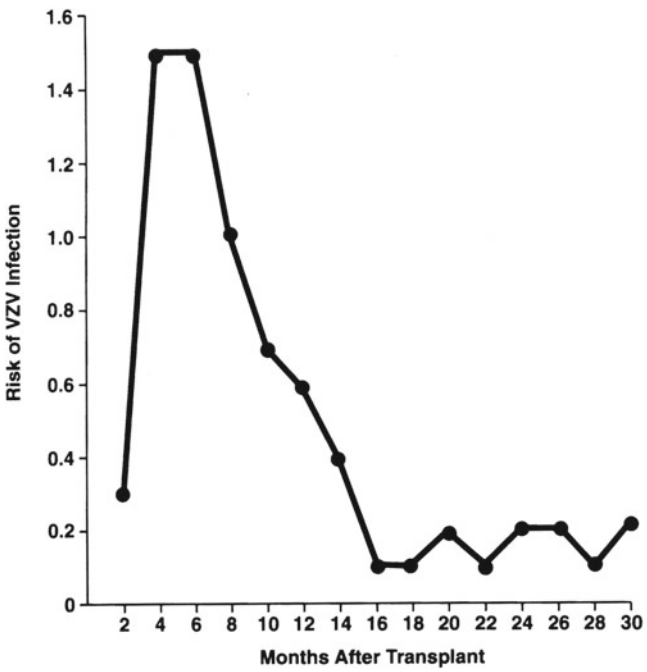

FIGURE 27-5. Risk of herpes zoster by month after BMT expressed as incidence per patient-day (%). (Modified from Locksley RM, Flournoy N, Sullivan KM, et al. Infection with varicella-zoster virus after marrow transplantation. J Infect Dis. 1985;152:1172–1181, by permission of Oxford University Press).

Clinical herpes zoster in transplant patients can present in one of three patterns. In most instances, the patient will develop a typical dermatomal skin eruption similar to that seen in immunocompetent patients, although the risk of dissemination is higher. Malaise and neuralgic pain in the involved dermatome precede onset of the rash by hours to a few days. A small number of patients present with a prodrome of fever, abdominal pain, nausea, and vomiting [187, 189, 190]. In 195 BMT patients with zoster, the rash involved the following dermatomes: cranial, 16 %; cervical, 17 %; thoracic, 47 %; lumbar, 21 %; and sacral, 12 % [187]. Multiple or noncontiguous dermatomes are involved more frequently in transplant recipients than immunocompetent hosts. In untreated immunocompetent patients, cessation of new vesicle formation occurs in about 3–5 days and lesion healing requires 2–3 weeks; in immunocompromised patients, these intervals are 8 days and 3–4 weeks, respectively. Prior to antiviral therapy, transplant patients sometimes developed indolent lesions that persisted for weeks or months and resolved only when immunosuppression therapy was reduced. Complications related to dermatomal herpes zoster may include postherpetic neuralgia (20–35 %), cutaneous scarring (19 %), ocular complications following trigeminal zoster, and bacterial superinfection (17 %) [133, 145, 150, 154, 155, 187, 191, 192]. Second zoster episodes of occur in 5–15 % of transplant patients [155].

Cutaneous VZV dissemination occurs in 15 % to 30 % of HCT patients who initially present with dermatomal herpes zoster, with higher rates among allogeneic HCT patients [145, 151, 187]. Cutaneous dissemination does not increase mortality rate, but predicts possible visceral disease, including encephalitis [193–197], myelitis, cranial neuropathies [198–201], pneumonitis, hepatitis [202], and necrotizing retinitis [203, 204]. Approximately one third of HCT patients with dermatomal zoster and cutaneous dissemination subsequently develop visceral complications.

In HCT patients, 15–20 % of VZV reactivations consist of disseminated varicella-like skin lesions with no obvious primary dermatomal eruption, a syndrome termed *atypical generalized zoster*. This probably represents reactivation of endogenous latent VZV in a patient with no effective immune responses, although a second primary infection following reexposure to exogenous VZV may occur. This syndrome resembles varicella, with a 40–50 % risk of visceral involvement [151, 187]. Mortality due to VZV visceral dissemination was at >50 % in the pre-antiviral era, but is <15 % with effective treatment [205].

Visceral VZV disease with no cutaneous involvement, an uncommon presentation, is difficult to diagnose and carries a high mortality rate. HCT (and rarely SOT) patients present with fever, severe abdominal pain, nausea and vomiting, and gastrointestinal bleeding [190, 206–210]. Rapidly rising transaminases suggest acute necrotizing hepatitis, warranting

empiric intravenous acyclovir therapy. Inappropriate antidiuretic hormone secretion has been observed with this syndrome [206, 211]. In a series of ten HCT patients with visceral VZV, mean time from transplantation to onset was 153 days (range 60–280 days) [212]. At laparotomy or autopsy, these patients have ulcerations in the gastrointestinal tract; hemorrhagic necrosis in the liver, pancreas, and kidneys; and positive VZV cultures and PCR from blood and involved organs [211, 213].

Lastly, patients may develop subclinical reactivation of latent VZV (due to boosted humoral and cellular immune responses). In some HCT patients, subclinical VZV reactivation has been documented by PCR detection of VZV DNA in circulating mononuclear cells, accompanied by restoration of lymphocyte proliferation responses to VZV antigens [172]. Patients with subclinical reactivation are at low risk for subsequent zoster. A related group of patients has been described who developed neuralgic pain typical of zoster, but no cutaneous lesions (termed *zoster sine herpete*), followed by a boost in VZV-specific humoral and cellular immune responses [214].

Prior to availability of effective antiviral therapy, the mortality rate for zoster in transplant populations was about 10 %, with deaths due to visceral dissemination, especially pneumonitis [215]. In a review of 195 BMT patients, mortality associated with dermatomal zoster and varicella-like syndrome was 6.7 % and 28 %, respectively [187]. In a more recent study of 43 acyclovir treated episodes in autologous BMT patients, all patients survived, including ten patients with varicella-like syndrome [151]. In a study of 25 liver transplant patients with zoster (1993–2004), only eight (29 %) were hospitalized, 16 (55 %) were treated with oral antivirals alone, and none developed visceral involvement [183].

27.4.5 Diagnosis

The appearances of classical varicella and herpes zoster are distinctive. In most cases, a clinical diagnosis is accurate and reliable. However, in transplant patients, presentations can be atypical and distinguishing between VZV and HSV disease can sometimes be difficult. When there is diagnostic uncertainty or visceral disease is suspected, laboratory confirmation is required.

Unlike HSV or CMV, VZV is not shed asymptomatically. Therefore, identification of VZV virions, antigens, or nucleic acids from cutaneous lesions or tissue biopsies is diagnostic of active infection. VZV can be cultured by inoculation of vesicular fluid or tissue extracts into monolayers of human fetal diploid kidney or lung cells. VZV is labile and efforts should be made to minimize specimen transport and storage time. Characteristic cytopathic effects are seen in tissue culture within 3–7 days. VZV identification can be confirmed by direct immunofluorescent staining of infected cells using VZV-specific monoclonal antibodies. A more rapid diagnosis can be made by using direct fluorescent antibodies (DFA) to detect VZV glycoprotein antigens in cellular scrapings obtained from the base of a fresh vesicle when the clinical presentation is atypical [216, 217].

PCR is useful for detecting VZV DNA in skin lesions, tissues or fluids (e.g., cerebrospinal fluid) and is much more sensitive than culture [218]. PCR of blood is the diagnostic method of choice for patients with visceral disease, including patients without cutaneous involvement [219–221].

Serologic assays help determine patient susceptibility to VZV but are not useful for diagnosing acute infections. Most laboratories use an ELISA method that can detect either IgG or IgM. Antibodies appear several days after onset of varicella and peak at 2–3 weeks. Patients with zoster are typically VZV-seropositive at the time of disease onset, but most show a significant rise in titer during the convalescent phase. Serologic boosting only provides retrospective confirmation of the diagnosis.

27.4.6 Therapy

The availability of safe and effective antiviral drugs has greatly reduced the high mortality rate previously associated with VZV disease in transplant patients. Controlled trials of intravenous acyclovir in immunocompromised patients with varicella demonstrated a dramatic reduction in complications, especially pneumonitis. Therapy should be initiated immediately with intravenous acyclovir at 10 mg/kg every 8 h, though some experts recommend higher doses of 12–15 mg/kg every 8 h (adjusted for renal function, as necessary). A switch to oral acyclovir, valacyclovir, or famciclovir, can be considered when the patient is afebrile and new lesion formation has ceased, to complete 10–14 days of therapy. In a retrospective review of 14 pediatric heart transplant patients with varicella, half were treated with intravenous acyclovir and half received oral valacyclovir; all patients recovered without serious complications [222]. Among 61 pediatric liver transplant patients with chickenpox, 95 % were treated with oral antiviral therapy with no evidence of visceral dissemination [223]. When feasible, immunosuppressive treatment should be temporarily reduced in transplant patients with varicella. If the patient is hospitalized, isolation procedures are essential to prevent nosocomial transmission.

Intravenous acyclovir (dosed as above) remains the therapy of choice for zoster in severely immunocompromised patients, including allogeneic HCT patients within 4 months of transplantation, HCT patients with moderate-to-severe acute or chronic GVHD, any patient requiring potent antirejection therapy, or any patient with suspected visceral dissemination. Studies in BMT patients with zoster proved that acyclovir was highly effective at preventing visceral dissemination [224, 225]. Since most VZV-related fatalities result from disseminated infection, treatment with acyclovir has

markedly reduced the herpes zoster mortality rate in transplant patients. Intravenous acyclovir is also the drug of choice for treating disseminated disease. When disease is well-controlled, the patient can be switched from intravenous to oral antiviral medication to complete therapy. For less severely immunosuppressed patients, including most SOT and autologous HCT patients with zoster, oral therapy with acyclovir (800 mg five times daily), valacyclovir (1000 mg t.i.d.), or famciclovir (500 mg t.i.d.), coupled with close clinical observation, is reasonable [226]. Patients should be treated until healing is complete (or a minimum of 10–14 days) to reduce risk of relapsing disease. Herpes zoster involving the first division of the trigeminal nerve (herpes zoster ophthalmicus; HZO) carries a substantial risk of ocular complications. Patients who present with HZO should initially be treated with intravenous acyclovir and evaluated promptly by an ophthalmologist [227, 228].

Since zoster tends to occur late after transplantation, most patients have been discharged from the hospital when the disease develops. Treating shingles in transplant patients on an outpatient basis with oral antiviral drugs is an attractive approach, although data from controlled studies are limited. In one study, 27 allogeneic BMT patients with zoster were randomized to receive oral or intravenous acyclovir [229]. No VZV dissemination occurred in either group, and no differences in healing or clinical outcome were apparent. Published data from clinical trials with famciclovir and valacyclovir for herpes zoster in immunocompromised patients remain limited, but a large body of clinical experience suggests these drugs are safe and effective and can replace oral acyclovir [230, 231].

Resistance to acyclovir occurs less frequently with VZV than with HSV and occurs more frequently in patients with AIDS than in transplant recipients [232, 233]. The possibility of acyclovir-resistant virus should be considered in immunocompromised patients who have progressive VZV disease despite appropriate antiviral therapy Based on anecdotal experience, foscarnet is recommended for management of disease caused by acyclovir-resistant VZV [234].

27.4.7 Prophylaxis

Antiviral prophylaxis in the posttransplant period has the dual advantage of effectively preventing both HSV and VZV disease. Results from two trials of 6 months of acyclovir prophylaxis in BMT patients showed zoster in 11 of 62 placebo recipients (18 %) and in none of 62 acyclovir-treated patients [112, 235]. In subsequent studies, oral acyclovir was extended to 12 months and the risk of zoster was reduced throughout the prophylaxis period [118, 143, 236]. These studies revealed no negative impact of acyclovir therapy on marrow engraftment, organ rejection or VZV-specific immune reconstitution. Zoster incidence increased after prophylaxis was discontinued. For populations receiving prophylaxis for 6 months, the cumulative number of zoster cases at 12 months was identical between acyclovir and placebo groups [112, 126, 235, 237, 238]. In HCT patients receiving 12 months of prophylaxis, the highly significant difference in zoster risk seen at 12 months was not present at 24 months after randomization [143]. Risk factors for zoster after prophylaxis termination included continued immunosuppression and HLA-mismatch donor status. However, in larger, more contemporary studies, “rebound” herpes zoster following 12 months of acyclovir prophylaxis was not observed [239, 240]. Importantly, acyclovir prophylaxis (e.g., 400–800 mg p.o. bid) effectively prevents herpes zoster during the early posttransplant period when patients are most severely immunosuppressed and are at highest risk for VZV-related complications. Because of its superior pharmacokinetic properties, many experts recommend valacyclovir (e.g., 500 mg p.o. daily or bid) as an alternative [241, 242]. Famciclovir would likely also be efficacious for prophylaxis, but supporting data are lacking.

Prophylactic antiviral drug regimens (e.g., valganciclovir) intended to prevent CMV disease in transplant patients effectively prevent herpes zoster [106, 144, 147, 149, 159, 243, 244]. However, some studies have shown that SOT patients receiving only preemptive therapy for CMV have a higher incidence and shorter time to onset of herpes zoster when compared with patients receiving daily prophylaxis [245]; other studies do not support this observation [246]. Daily acyclovir or valacyclovir should be considered in the early posttransplant period for patients whose CMV risk is managed with preemptive rather than prophylactic therapy.

Recommendations for duration of HSV/VZV-specific prophylaxis following HCT have evolved [239, 244, 247]. In 2009, a CDC panel published guidelines recommending acyclovir prophylaxis for 1 year following HCT, due to proven efficacy and safety [248]. Continuing prophylaxis beyond 1 year does result in continued reduction of herpes zoster risk in HCT patients and can be considered for those patients with continued need for systemic immunosuppression or with chronic GVHD [239, 248]. In all transplant patients, resumption of prophylaxis should be considered during intensification of immunosuppressive therapy, a period of enhanced risk [249].

A recent investigation was undertaken to determine if allogeneic HCT recipients managed with reduced-intensity conditioning (RIC) could safely forego routine antiviral prophylaxis [250]. The cumulative 2-year incidence of zoster was 20.7 % and did not differ between patients receiving myeloablative conditioning or RIC; 29 % of the zoster patients developed complications [250]. Therefore, antiviral prophylaxis is still warranted even in the setting of non-myeloablative conditioning regimens [149].

27.4.8 Prevention

27.4.8.1 Post-Exposure Prophylaxis

Serological screening prior to transplantation to determine susceptibility to VZV is essential to guide decision-making regarding immunoprophylaxis. Transplant patients (especially VZV-seronegative patients) should avoid exposure to persons with active VZV infection. Susceptible transplant patients with a close exposure to VZV (either chickenpox or zoster) should receive varicella-zoster immune globulin to provide passive immunity. VariZIG™ (Cangene Corporation, Winnipeg, Canada), the varicella-zoster globulin product available in the USA since 2006, can be ordered on a patient-specific basis from FFF Enterprises, Temecula, CA (24-h hotline: 1-800-8437477). In most cases, varicella-zoster immune globulin administration will not prevent infection in the susceptible host, but will significantly reduce illness severity [185]. Placebo-controlled trials in high-risk immunocompromised children demonstrated that varicella-zoster immune globulin ameliorated severity of chickenpox. A single treatment will reduce the risk of disseminated infection by about 75 % and provide 4 weeks of passive immunity. Controlled studies of post-exposure immunoprophylaxis have not been conducted in transplant populations and current recommendations are extrapolated from the pediatric data [251]. For maximal efficacy, varicella-zoster immune globulin must be administered as soon as possible after exposure (optimally within 96 h, but as late as 10 days may still provide benefits) [252]. Standard intravenous immunoglobulin (IVIG) contains substantial amounts of VZV-specific IgG and can be used if varicella-zoster immune globulin is not available. In situations where immune globulin is not available, post-exposure chemoprophylaxis with acyclovir or valacyclovir should be considered in seronegative or high-risk transplant patients who have been exposed to VZV [177, 248, 253, 254]. Some experts recommend combination prophylaxis with varicella-zoster immune globulin plus antiviral therapy for all VZV-seronegative HCT patients exposed to VZV [177]. Varicella-zoster immune globulin is not useful for treatment of established varicella or herpes zoster. Post-exposure vaccination, the intervention of choice in immunocompetent persons, is not recommended in transplant patients.

27.4.9 Vaccination

27.4.9.1 Varicella Vaccine

A more attractive option for prevention of VZV infection and disease is vaccination [255–259]. The VZV_{Oka} live-attenuated virus varicella vaccine (Varivax®) has been routinely administered to immunocompetent children in many countries for 20 years [260]. Concerns about use of live-attenuated virus vaccines in immunocompromised patients have focused on the potential for the vaccine virus to cause disease and that immunocompromised patients may fail to mount a protective immune response [261–263]. Preliminary data suggest that varicella vaccine is safe and effective when given prior to SOT [223, 264–269]. In a study conducted in France, all VZV-seronegative renal transplant candidates were given a single dose of the varicella vaccine; patients who failed to seroconvert received a second dose [264]. Following transplantation, varicella incidence was 12 % among vaccinees and 45 % among unvaccinated patients with no history of chickenpox. Varicella was less severe in the vaccinated population (no deaths) than in the seronegative unvaccinated population (three deaths). Zoster developed in 13 % of the patients with a history of varicella, in 7 % of the vaccine recipients, and in 38 % of the seronegative unvaccinated patients who developed primary varicella after transplantation [264]. When feasible, two doses of vaccine should be administered with a minimum interval of 4 weeks, and completed at least 2 weeks (preferably 4 weeks) prior to transplantation. VZV-seronegative household members should be vaccinated to minimize exposure of the transplant recipient to wild-type virus [248, 270].

An alternative approach is to administer primary or booster varicella vaccination after organ transplantation [142]. Pediatric SOT patients who received varicella vaccine demonstrated good humoral and cellular immune responses with few adverse effects [251, 271–274]. In similar studies involving pediatric HCT patients, administration of live-attenuated varicella vaccine after transplantation resulted in seroconversion and reduced rates of varicella [275, 276]. Posttransplant varicella vaccination of adults has not been studied [258]. Although further research is necessary, vaccination will likely play an increasingly important role in protecting transplant patients from varicella. Compared with varicella-zoster immune globulin or treatment of varicella, immunization would be highly cost-effective [277].

27.4.9.2 Herpes Zoster Vaccine

Over 90 % of adult transplant patients are latently infected with VZV and at risk for zoster [257, 268]. A VZV_{Oka}-containing vaccine (Zostavax®) reduces the incidence and severity of herpes zoster in immunocompetent adults, but has not been fully evaluated in immunocompromised populations [278, 279]. Small studies of live, attenuated VZV_{Oka} vaccine administration (usually >1 year after transplantation) for prevention of zoster in adult autologous HCT recipients have been reported [280, 281]. The vaccine was well tolerated and immunogenic in selected immunologically stable patients, but much larger studies are required to assess safety and efficacy [282]. Pretransplant administration of herpes zoster vaccine to SOT candidates should theoretically be safe and is being evaluated in ongoing trials.

Due to concerns regarding use of live-virus vaccines after HCT, a novel approach to preventing herpes zoster is the use

of a non-replicating VZV vaccine to boost cellular immune responses and prevent reactivation. Randomized studies have been performed that compared heat-inactivated VZV vaccine or placebo administered after HCT transplantation. Compared with the placebo group, vaccine recipients had a lower rate of zoster [283] and significantly reduced disease severity [284]. The level of protection correlated with the level of reconstitution of $CD4^+$ T-cell immunity against VZV. Recently, an adjuvanted subunit herpes zoster vaccine containing VZV glycoprotein E has shown to be highly effective for preventing shingles in immunocompetent adults [285]. While studies in transplant populations have not yet been reported, this vaccine avoids the risk associated with replicating vaccine virus and has great promise for use in immunocompromised patients.

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28
Human Herpesvirus-6, -7, and -8 After Solid Organ Transplantation

Nina Singh

Since 1986, three novel herpes viruses, human herpesvirus (HHV)-6, -7, and -8, have been discovered. Kaposi sarcoma herpesvirus is causally associated with all forms of Kaposi sarcoma, including the type seen after transplantation. The role of HHV-6 and HHV-7 as pathogens in transplant recipients is less well understood. Although the complete spectrum of clinical sequelae and ultimately the effective prophylaxis and management of these viruses has yet to be fully elucidated, existing data suggest that these viruses are potentially pathogenic and clinically relevant in transplant recipients.

28.1 Human Herpesvirus-6

28.1.1 Biologic Features

HHV-6 belongs to the subfamily Beta herpesviridae in the genus Roseolovirus. HHV-6 is an enveloped virion with an icosahedral nucleocapsid of 162 capsomeres that contains a large, double-stranded DNA [1, 2]. Phylogenetically, HHV-6 is most closely related to HHV-7 and cytomegalovirus (CMV); nucleotide sequencing shows 66% DNA sequence homology between CMV and HHV-6 [3].

The primary target cell for HHV-6 is $CD4^+$ T lymphocytes, a characteristic that HHV-6 shares with human immunodeficiency virus (HIV) [4, 5]. The propensity to infect $CD4^+$ T cells preferentially differentiates HHV-6 from other DNA viruses. The virus utilizes CD46 as a cellular receptor; however, role of a cofactor has been suggested given that some human T-cell lines are not permissive of the virus despite high expression of CD46. The cellular host range of HHV-6 is broad and includes not only $CD4^+$ T cells but also $CD8^+$ T lymphocytes, NK cells, macrophages, megakaryocytes, glial cells, and epithelial cells [6]. HHV-6 has immunomodulatory characteristics; virus-infected peripheral blood mononuclear cells have been shown to demonstrate reduced cellular proliferation [7]. HHV-6 also modulates host immune response by altering cytokine and chemokine responses and by promoting a shift of T-helper responses from Th1 to Th2 phenotype [7–9].

Based on genomic DNA sequences, cell tropism, and protein expression, research studies have described two distinct variants of HHV-6 that have been designated as the A (HHV-6A) and B variants (HHV-6B) [10, 11]. The two variants exhibit 88% identity at the nucleotide level but differ in a number of their biologic properties. Most infections in transplant recipients are due to HHV-6B; the isolation of HHV-6A from blood samples is distinctly rare in transplant recipients. Lack of detection of HHV-6A may imply either that HHV-6A reactivation occurs infrequently or that it may be limited to sites other than the blood [12].

28.1.2 Transmission

Seroepidemiologic studies have shown that primary infection due to HHV-6 is usually acquired during the first year of life, with saliva the most likely mode of transmission. As with other herpesviruses, HHV-6 persists in the host in a latent form; seroprevalence in healthy adults exceeds 90% [13, 14]. Evidence of prior HHV-6 infection has been documented in 87–91% of the solid organ and 78–100% of the hematopoietic stem cell transplantation (HSCT) recipients [15]. Although the precise site of latency in the body is not known, the epithelial cells of the bronchial epithelia, salivary glands, and possibly monocytes/macrophages represent the most likely sites [16]. Neurons and glial cells may also be the sites where HHV-6 may be latent. A novel and an increasingly recognized form of HHV-6 persistence is integration of HHV-6 DNA sequences in the chromosomes of host peripheral blood mononuclear and other cells [17–20]. Chromosomal integration of HHV-6 DNA is a genetically inherited phenomenon and is considered to be responsible for vertical transmission of HHV-6 [21] and for the transmission of HHV-6 through stem cell transplantation [17]. This condition is present in ~1% of people and has been anecdotally implicated in HHV-6 central nervous system disease [22].

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Most infections in transplant patients are considered to result from reactivation of the latent virus. Donor transmission of HHV-6 with the transplanted allograft has also been documented. Mononuclear cells latently infected with HHV-6 in the donor allograft are believed to be the likely source of transmission. Following liver transplantation, primary HHV-6 infection has been reported in 61–100% of the patients who were seronegative for HHV-6 prior to transplantation [23, 24]. HHV-6 has been shown to develop latency in the kidney in vivo [25]. Indeed, two renal transplant recipients who received the allografts from the same cadaveric donor were documented to have identical genomic pattern of their HHV-6 isolates [26]. Transplanted allograft was the likely source of fatal infection due to HHV-6 variant A in a renal transplant recipient who was seronegative for HHV-6 prior to transplantation [27].

28.1.3 Epidemiology

Overall, 38–55% of the renal, 22–54% of the liver, 36% of the heart, and up to 57% of the lung or heart–lung transplant recipients develop HHV-6 infection [27–35]. Following living-related liver transplantation, HHV-6 infection has been documented in 48% of the patients [36]; these included all seronegative and 42% of the recipients seropositive for HHV-6 prior to living-related transplantation [36].

HHV-6 infections typically occur between 2 and 4 weeks after transplantation; this characteristic timing of onset distinguishes HHV-6 from other beta herpesvirus infections that usually occur posttransplantation [37]. In a study in liver transplant recipients where HHV-6 and HHV-7 DNA detection was sought in the plasma, HHV-6 infection occurred in 38% (15/40) patients; 67% of the infections occurred at 2 weeks posttransplantation after which the frequency of viral genome in the plasma sharply declined [36]. In contrast, only 31% of the HHV-7 infections were detected at 2 weeks with no distinct peak in time to detection. Further, HHV-7 DNA remained detectable in 10–20% of the patients until 8 weeks posttransplant [36]. Temporal sequence of infections with the three beta herpesviruses in organ transplant recipients showed that HHV-6 reactivation occurred at a median of 20 days, followed by HHV-7 (median 26 days) and CMV (median 36 days) [38].

Risk factors for HHV-6 infection in organ transplant recipients have not been fully defined. Receipt of OKT3 monoclonal antibodies or antithymocyte globulin has been associated with HHV-6 reactivation in solid organ transplant (SOT) recipients [27, 39]. HHV-6 seroconversion in one study was noted more frequently in patients who received immunosuppressive regimens containing sirolimus and IL-12 receptor antibodies as induction therapy [40]. There is conflicting data on the association of HHV-6 with rejection. HHV-6 infection has been shown to increase the expression of adhesion molecules and the number of human leukocyte antigen class II–positive T cells [41]. HHV-6 infection has been associated with acute allograft rejection in some, but not in all reports [30, 34, 38]. HHV-6 infection and peak HHV-6 viral load in liver transplant recipients were associated with only those rejection episodes that occurred after day 30 post-transplantation [42]. In renal transplant recipients, HHV-7, but not CMV or HHV-6, correlated with biopsy-proven cellular rejection [43]. An association between chronic allograft nephropathy and HHV-6 infection has been reported [44]. Histopathologic findings of chronic allograft nephropathy were observed in late biopsies in renal transplant recipients who had received antithymocyte globulin/ALG as induction therapy and had HHV-6 reactivation [45].

Liver transplant recipients with hepatocellular carcinoma in one report were more likely to develop HHV-6 viremia than patients without it [34]. The association between HHV-6 and hepatocellular carcinoma was considered to be mediated through hepatotropic viruses, for example, hepatitis B and C viruses [34]. It was proposed that hepatotropic viruses may facilitate the emergence of HHV-6 from latency by transactivating its immediate early proteins.

HHV-6 infections develop in 35–42% of allogeneic stem cell transplant recipients. Higher rates (87–92%) in cord blood transplant recipients are considered to be due to paucity of primed HHV-6 specific memory T cells and low ability of cord blood cells to produce protective cytokines [46, 47]. Risk factors for HHV-6 infection in HSCT recipients include younger age, transplantation for hematologic malignancy, HLA mismatch, and corticosteroid administration [46, 47].

28.1.4 Clinical Manifestations

The clinical sequelae of HHV-6 may result from symptoms directly attributable to the virus or from its immunomodulatory effects. Symptomatic infection due to HHV-6 occurs more frequently in HSCT recipients than it does in SOT recipients (Table 28-1). The most frequently observed

Table 28-1. Clinical sequelae of human herpesvirus-6

Supportive evidence from cohort studies
Encephalitis
Bone marrow suppression
Association with fungal infections
Association with cytomegalovirus infection
More aggressive recurrence of hepatitis C virus after liver transplantation
Evidence from case reports or case series
Pneumonitis
Exanthem
Hepatitis
Gastroduodenitis
Leukocytoclastic vasculitis
Proposed association with conflicting supportive evidence
Association with allograft rejection
Association with graft-versus-host disease
Mortality

clinical features of HHV-6 are skin rash and bone marrow suppression. Organ-specific involvement (e.g., interstitial pneumonitis, encephalitis, and hepatitis) is less common.

28.1.5 Direct Effects

A nonspecific febrile syndrome with or without a skin rash is attributed to HHV-6 in both HSCT and SOT recipients. The febrile mononucleosis syndrome attributable to CMV in transplant recipients may be related to concurrent infection with HHV-6 and HHV-7 rather than just CMV alone. HHV-6 is associated with acute and, less frequently, chronic myelosuppression [48]. In vitro studies have shown that colony-forming units of granulocyte–macrophage precursor decrease by 43% with HHV-6 infection; the growth of the multipotential precursor of granulocytes, erythrocytes, monocytes, and megakaryocytes was inhibited by 71%, and that of erythroid burst-forming units was reduced by 73% [9]. The most common hematologic sequelae of HHV-6 infection are delayed platelet engraftment and leukopenia. Cytokine-produced or virus-produced soluble factors may mediate the marrow suppressive effect of HHV-6 [9]. However, research findings have documented the direct effect of HHV-6 on the hematopoietic progenitor cells in vitro [49].

HHV-6 is a neurotropic virus. A number of well-documented case reports of HHV-6 encephalitis, as well as at least three studies that included concurrent controls, have documented an association between HHV-6 and central nervous system (CNS) complications of unidentifiable etiology [50–52]. In a report in liver transplant recipients, 15% (12 of 80) of the patients had mental status changes of unknown etiology after transplantation [34]. Patients with HHV-6 viremia had a significantly higher incidence of mental status changes of unidentifiable etiology (29%; 9 of 31), compared with those without HHV-6 viremia (6%; 3 of 49; $P=0.008$). Among 338 bone marrow transplant (BMT) recipients, CNS symptoms developed in 24 (7.1%). Researchers detected HHV-6 DNA in the cerebrospinal fluid (CSF) in 23% (5 of 22) of the patients with CNS symptoms [51]. None of the five cases in whom HHV-6 DNA was detected in the CSF samples had an identified cause of their CNS symptoms, and none of the 11 cases with a known etiology of CNS symptoms had HHV-6 DNA in the CSF ($P=0.03$). Allogeneic BMT recipients who received CD3-specific monoclonal antibody (anti-CD3) were more likely to develop encephalitis than were those patients who did not receive anti-CD3 ($P=0.008$) [53]. After adjusting for anti-CD3 therapy, HHV-6 was significantly associated with encephalitis ($P=0.009$) [53].

Mental status changes ranging from confusion to coma, seizure, and headache are the predominant clinical manifestation of HHV-6. Focal neurologic findings are rare. CSF pleocytosis ranging from 6 to 53 cells/mL was present in 50% of the patients with HHV-6 encephalitis in one review [52]. In the same review, neuroimaging abnormalities were present in two out of eight patients on magnetic resonance imaging [52]. These abnormalities included multiple, nonenhancing, low-attenuation lesions in the gray matter. More recently, HHV-6 has been identified as an etiologic agent for posttransplant acute limbic encephalitis, a distinct syndrome characterized by seizures, dense anterograde amnesia, and neuroimaging abnormalities showing low-attenuation lesions typically involving bilateral medial temporal lobes [54, 55].

Some but not all studies in HSCT recipients have suggested a causal association between HHV-6 and graft-versus host disease) (GVHD [56–58]. HHV-6 may present as an exanthem that may be indistinguishable clinically from GVHD; skin biopsy with demonstration of HHV-6 by immunohistochemistry and polymerase chain reaction (PCR) may be diagnostically useful in differentiating the two conditions or showing their coexistence [56, 58].

Case reports in stem cell transplant recipients have documented an association between obliterative bronchitis and HHV-6 [59, 60]. Although HHV-6 has been detected in 9.4–14.6% of the bronchoalveolar lavage fluid samples in lung transplant recipients [33, 61], its relevance as a pathogen in this setting is controversial. HHV-7, on the other hand, was proposed to have a role in the pathogenesis of bronchiolitis obliterans with organizing pneumonia (BOOP) given that HHV-7-DNA was detected in all (7/7) transbronchial biopsies that showed BOOP while coinfection with other beta herpesviruses was rare [61]. Rare cases of gastrointestinal disease, hepatitis, and hemophagocytic syndrome due to HHV-6 have also been documented in transplant recipients [30, 62–64].

28.1.6 Indirect Sequelae

HHV-6 has been described as an immunomodulatory and immunosuppressive virus that may facilitate superinfections with other opportunistic infections in transplant recipients, particularly CMV [7, 23, 37, 48]. A study identified primary HHV-6 infection as a significant risk factor for the development of symptomatic CMV infection, including tissue invasive CMV disease in liver transplant recipients [23]. In renal transplant recipients at risk for primary CMV infection, HHV-6 infection is significantly associated with the development of CMV viral syndrome and CMV hepatitis [48]. HHV-6 infection is an independently significant predictor of invasive fungal infections in liver transplant recipients. When the risk of opportunistic infections is controlled for the level of immunosuppression, HHV-6 infection increases by 3.68-fold in liver transplant recipients [42]. Another study reported that liver transplant recipients with HHV-6 had a significantly higher mortality; the independent association between HHV-6 and late mortality approached statistical significance in that study [34].

HHV-6 infection has been shown to have a role in the pathogenesis of hepatitis C [42, 65]. Although HHV-6 viremia did not affect the overall rate of recurrence of hepatitis C, it was associated with a more severe form of recurrence [42]. Patients with HHV-6 viremia tended to have an earlier recurrence and higher fibrosis scores on recurrence than did those with HHV-6 viremia [65]. Of note, patients who received ganciclovir as preemptive therapy for CMV infection had lower total Knodell scores and a trend toward lower fibrosis scores than those of patients who did not receive ganciclovir [65]. A protective effect of ganciclovir on the severity of HCV recurrence was proposed to be mediated via its mitigating effect on HHV-6 infection [65].

Although the pathophysiologic basis of the association between HHV-6 and severity of HCV, particularly the progression of fibrosis remains to be determined, a number of biologic plausibilities exist. HHV-6 is a potent inducer of cytokines, e.g. TNF-α, that play a role in the development of hepatic fibrosis. It is proposed that TNF-α leads to activation of Kupffer cells in the liver, a key component in the cascade of hepatic fibrogenesis [66]. Production of TNF-α can also lead to the induction of transforming growth factor (TGF-β1), a fibrogenic cytokine that is a potent stimulus for hepatic stellate cells to increase the production of extracellular matrix protein that ultimately results in hepatic fibrosis [67].

Clinical relevance of chromosomally integrated HHV-6 has not been fully defined in transplant recipients. Chromosomally integrated HHV-6 was documented in ~1% of the organ transplant recipients and correlated with an insignificantly higher risk of allograft rejection compared with patients who had low grade or no HHV-6 viremia [68].

28.1.7 Diagnosis

Although viral inclusion–bearing cells may appear in histopathologic samples [48], HHV-6 characteristically elicits little inflammatory response. Multinucleated giant cells, which are similar to the cytopathogenic effect caused by HHV-6 in human T lymphocytes in vitro, and enveloped virions with a prominent tegument that can by visualized by electron microscopy in the tissue have been proposed as morphologic criteria when assessing the possibility of tissue-invasive HHV-6 disease [69].

Serologic, virologic, and in situ immunohistochemistry assays have been used for the diagnosis of HHV-6. For the serologic diagnosis of HHV-6, enzyme immunoassays are more sensitive than are the fluorescence assays [70]. As with all herpesviruses that establish latency, serologic tests may not be reliable indicators of active infection, although they are useful for the determination of seroprevalence. Antigenic cross-reactivity or concomitant infection with other herpesviruses may confound the specificity of the serologic assays or the interpretation of changes in HHV-6 antibody titers. Immunoglobulin M (IgM) per se is also not a reliable marker for HHV-6 infection because most cases confirmed by culture or seroconversion have no detectable IgM [70]. Furthermore, up to 5% of healthy adults demonstrate IgM positivity at any time [70].

HHV-6 induces a characteristic cytopathic effect in primary lymphocyte culture with “large ballooning” refractile cells and the loss of normal lymphocyte clumping. The detection of HHV-6 in cell culture, however, must be confirmed by HHV-6–specific reagents and not merely by the cytopathic effect. HHV-6 isolation in cell culture is labor-intensive and time-consuming, requiring from 5 to 21 days for detection. A rapid shell vial (early antigen) assay can detect HHV-6 within 72 h [48]. This assay is analogous to the shell vial assay for the diagnosis of CMV. Compared with conventional cell culture, the assay has a sensitivity of 86% and a specificity of 100% in BMT and liver transplant recipients [71].

The ability of qualitative PCR to detect latent virus limits its use for the diagnosis of HHV-6. Latently infected peripheral blood mononuclear cells, however, contain fewer than ten HHV-6 genomes per 106 cells. Nevertheless, PCR has other advantages. PCR positivity in cell-free specimens can be diagnostically useful. Furthermore, HHV-6 variant discrimination is readily accomplished by PCR. The blood compartment used for the detection may influence the yield of the virus. Whereas HHV-6B DNA is detectable in both peripheral blood leukocytes and plasma, HHV-6A DNA is detected primarily in the plasma according to one report [72].

A potential caveat is that patients with chromosomally integrated HHV-6 may have detectable virus in the CSF or serum even in the absence of active infection [17]. Antigenemia or viral isolation may have diagnostic utility in such cases [17]. A decline in viral load in temporal association with treatment also suggests a causal role of HHV-6 in patients with a clinical illness compatible with HHV-6 [17–19].

Immunohistochemical stains for detecting HHV-6 in formalin-fixed, paraffin-embedded tissue are also available. Immunohistochemical staining of tissues with murine monoclonal antibody that is reactive against both the structural protein p101 of variant B and the structural protein glycoprotein 82 (gp82) of variant A detects cells that are productively, and not latently infected with HHV-6.

28.1.8 Management

28.1.8.1 Prevention

Of currently available antiviral agents, acyclovir has consistently shown poor activity against HHV-6 in vitro [73]. Ganciclovir, on the other hand, has anti-HHV-6 activity with EC_{50} ranging from 0.56 to >25 in various studies. HHV-6 UL69 is the functional homolog of human CMV

UL97-encoded kinase that converts ganciclovir to its monophosphate metabolite in the infected cell [73]. In vitro studies have shown that the capacity of HHV-6 UL69 phosphorylates ganciclovir is 10-fold lower as compared to that of human CMV UL97 [74]. Furthermore, the affinity of ganciclovir triphosphate for HHV-6 DNA polymerase is 6-fold less than that for CMV and 800-fold less compared to acyclovir triphosphate for herpes simplex virus (HSV) DNA polymerase [75].

Antiviral prophylaxis with ganciclovir has been shown to be protective against HHV-6 infection in some but not all studies. In HSCT recipients, HHV-6 infection was documented in 39% (11/28) of the patients who did not receive ganciclovir compared to 0/13 in those who did [74]. In renal transplant recipients receiving ganciclovir HHV-6, viremia appeared later after transplantation (42 vs. 21 days) and was shorter in duration (29 vs. 62 days); however, there was no difference in the incidence of viremia (71% vs. 61%) [76]. Low-grade viremia was documented in ~14% of the organ transplant recipients receiving valganciclovir for 100 days posttransplant; however, no clinical manifestations could be attributed to it [77]. In contrast, suppression of CMV was complete. Thus, antiviral prophylaxis is not consistently effective in the prevention of HHV-6. The role of targeted prophylaxis based on periodic monitoring for HHV6 viremia has also not been defined. Given lack of standardized quantitative PCR cutoffs that can reliably predict disease, and erratic efficacy of currently available antiviral agents against HHV-6, a preemptive therapeutic approach cannot be recommended at this time.

28.1.8.2 Treatment

Overall mortality in patients with HHV-6 meningoencephalitis has ranged from 45% to 58% [50, 51]. Both ganciclovir and foscarnet have been successfully used for the treatment of meningoencephalitis, although outcomes have not been uniformly good. Of 13 HSCT recipients with HHV-6 encephalitis, 46% (6) died, that included four treated with ganciclovir and two who received ganciclovir and foscarnet [78]. In a review of HHV-6 encephalitis in transplant recipients, cure was documented in 7/8 patients who received ganciclovir or foscarnet for at least 7 days as compared to 0/4 who did not receive these drugs or received them for less than 7 days ($P=0.01$) [52].

There is evidence that antiviral activity against HHV-6 is different in serum and CSF. Amongst 11 HSCT recipients, median log decrease in the serum from 2.0 to 0 copies/mL and in the CSF from 4.4 to 2.0 copies/mL was documented with antiviral therapy [50]. Furthermore, decreases in CSF levels lagged behind that in the serum; earliest negativity in the CSF was observed at week 3 of antiviral therapy. Overall 5/11 patients died, including 4/5 who received foscarnet and ganciclovir [50]. In a recent report, ganciclovir and foscarnet were effective in inhibiting HHV-6 in peripheral blood mononuclear cells, but only foscarnet and cidofovir inhibited the virus in glial cells [79]. Rarely, ganciclovir-resistant HHV-6 disease has been documented in the setting of prolonged ganciclovir exposure [80]. It should be noted that while chromosomally integrated HHV-6 is not treatable with antiviral agents [17, 19], a case of severe encephalomyelitis due to chromosomally integrated HHV-6 successfully treated with foscarnet and ganciclovir has been reported [18].

Maribavir has been shown to be inactive against HHV-6 [73]. However, a recent study that evaluated the activity of maribavir against HHV-6 in a cell culture to slow the growth of lymphocytes documented that maribavir inhibits the replication of HHV-6 and the activity of UL69 protein kinase [81]. Other agents with diverse targets and enhanced activity are in various stages of development [82]. Hexadecyloxypropyl cidofovir or CMX001, is a lipid conjugate of cidofovir with in vitro activity against numerous double stranded DNA viruses. In vitro studies in propagated cell lines showed a 100-fold increase of cidofovir-diphosphate concentration in cells exposed to hexadecyloxypropyl cidofovir compared to cidofovir although clinical data are pending [83].

28.2 Human Herpesvirus-7

28.2.1 Overview

Infection due to HHV-7, like HHV-6, is ubiquitous. HHV-7, however, is more cell-associated, less lytic, and slower growing than HHV-6 [84]. HHV-7 not only exhibits selective tropism for $CD4^+$ T lymphocytes, but it also uses the CD4 molecule as its receptor. Primary infection due to HHV-7 also occurs during childhood, albeit at a slightly later age than does HHV-6. The salivary glands are believed to be the sites of persistence and replication of this virus.

HHV-7 may be a causative agent of roseola (exanthema subitum), particularly of the second attack. HHV-7 causes up to 10% of the cases of exanthema subitum. An association between HHV-7 infection and neurologic manifestations (e.g., acute hemiplegia in childhood and febrile convulsions) has been reported in nontransplant settings [85].

Viremia due to HHV-7 has been demonstrated by the detection of HHV-7 DNA via PCR of the peripheral blood mononuclear cells in 57% of the bone marrow and 39% of the renal transplant recipients [86, 87]. HHV-7 may be a cofactor in the pathogenesis of CMV in transplant recipients. Patients with CMV disease are more likely to have HHV-7 DNA detection than are those with asymptomatic CMV infection [57]. In a study of renal transplant recipients, patients coinfected with CMV and HHV-7 were more likely to have CMV disease, as compared to those with CMV infection only [43]. The febrile syndrome associated with CMV in liver transplant recipients may be due to concurrent infection with HHV-6 and/or HHV-7 [88]. A possible association between HHV-7 with bronchiolitis obliterans and organizing

pneumonia was reported in a lung transplant recipient [61]. One report of fatal encephalitis in association with HHV-7 in a peripheral blood stem cell transplant recipient has been published [89].

28.2.2 Management

Conflicting reports exist regarding the in vitro susceptibility of HHV-7 to ganciclovir. Researchers have evaluated the inhibitory activity of four classes of antiviral agents against HHV-7 [90]. These included foscarnet; the β-guanine analogues, acyclovir, ganciclovir, and penciclovir; the acyclic nucleoside phosphonates e.g., cidofovir and its cyclic derivatives, cyclic HPMPC [(S)-1-(3-hydroxy-2-phosphonylmethoxypropyadenine)]; and a series of benzimidazole ribonucleosides [90]. In this study, HHV-7 was most sensitive to cidofovir and its related compounds and least sensitive to the β-guanine analogues, including ganciclovir [90]. In another study, however, ganciclovir was shown to be highly active against HHV-7 [91].

Data regarding the clinical efficacy of antiviral agents for HHV-7 are sparse. One study reported clearance of HHV-7 DNA in the blood, as detected by PCR, in one of two BMT recipients who received ganciclovir and in two of those who received foscarnet [86]. A study of renal transplant recipients, however, showed that neither oral nor intravenous ganciclovir had an effect on the prevalence of HHV-7 viremia, as detected by PCR [92].

28.3 Human Herpesvirus-8 or Kaposi Sarcoma Herpesvirus

28.3.1 Biologic Features

Kaposi sarcoma herpes virus (KSHV) is a member of the subfamily Gammaherpesviridae that belongs to the genus Rhadinovirus. These herpesviruses are transforming viruses that are capable of causing tumors in their natural hosts. KSHV is most closely related to herpesvirus saimiri with which it bears 51% DNA sequence homology [93].

KSHV is unique among herpesviruses in that it contains an unprecedented number of genes that are transduced from the host cellular genomes during its evolution, a phenomenon known as molecular piracy [94]. While they are less important for viral replication, these genes do encode for cellular homologs that induce angiogenesis, regulate antiviral immunity, and alter cellular growth [94]. Spindle cells are the histopathologic hallmark of Kaposi sarcoma (KS). Most of these cells stain positive for endothelial cell markers; however, some cells express proteins that are characteristic of smooth muscle cells, macrophages, or dendritic cells. These data suggest that KS spindle cells are derived from a pluripotent mesenchymal progenitor cell [94].

Whether KS is a true malignancy or a cytokine-driven hyperplasia is unknown. KSHV is a potent inducer of angiogenic cytokines, such as IL-6, basic fibroblast growth factor, and IFN-γ [94]. KS spindle cells produce IL-6, and the addition of exogenous IL-6 to culture can enhance the proliferation of KS cells in culture, which has led many to believe that KS is a cytokine-driven lesion.

28.3.2 Epidemiology and Clinical Manifestations

The incidence of KS in transplant recipients largely parallels the geographic seroprevalence of KSHV in that region (Table 28-2). Consequently, wide geographic variations in KS rates are observed. In areas of low seroprevalence of KSHV (e.g., United States), KS affects less than 1% of the transplant recipients, and it accounts for 3–10% of all malignancies in transplant recipients. On the other hand, in South Africa and Saudi Arabia, KS affects up to 5% of the transplant recipients, and it accounts for between 59% and 87% of the posttransplantation malignancies [95, 100–102]. The striking male predominance of KS lesions in the nontransplant setting is less pronounced in transplant recipients, who have a male-to-female ratio of 3:1.

Although most KS lesions in transplant recipients result from the reactivation of latent virus, transmission via the transplanted allograft can occur. In a liver transplant recipient with KS, the anatomic distribution of KS in an autopsy study and the HLA haplotyping suggested that KS arose in the stromal endothelial cells of the donor liver [103]. After transplantation, the seroconversion rates range from 2% to

TABLE 28-2. Geographic variations in the incidence of Kaposi sarcoma in solid organ transplant recipients

Country	Incidence of Kaposi sarcoma (%)	Proportion of all malignancies in transplant recipients that are due to Kaposi sarcoma (%)
United States	0.5	3–10
France	0.6	8.3
Italy	1.6	–
Israel	2.4	–
South Africa	4	59
Saudi Arabia	5.3	87

Data from Moosa MR, Treurnicht FK, van Rensburg EJ, et al. Detection and subtyping of human herpesvirus-8 in renal transplant patients before and after remission of Kaposi's sarcoma. Transplantation. 1998;66:214–8 [95]; Penn I. Incidence and treatment of neoplasia after transplantation. J Heart Lung Transplant. 1993;12:S328–36 [96]; Harwood AR, Osoba D, Hofstader SL, et al. Kaposi's sarcoma in recipients of renal transplants. Am J Med. 1979;67:759–65 [97]; Hiesse C, Kriaa F, Rieu P, et al. Incidence and type of malignancies occurring after renal transplantation in conventionally and cyclosporine-treated recipients: analysis of a 20-year period in 1600 patients. Transplant Proc. 1995;27:972–4 [98]; and Montagnino G, Bencini PL, Tarantino A, et al. Clinical features and course of Kaposi's sarcoma in kidney transplant recipients: report of 13 cases. Am J Nephrol. 1994; 14:121–6 [99].

12% [104, 105]. Higher rates of seroconversion are observed in liver transplant recipients than in renal transplant recipients [105]. Another study indicated that seroconversion occurred at a mean of 5 months after transplantation and that it preceded KS by 11.5 months [100]. KS is one of the earliest posttransplantation malignancies to occur in transplant recipients. The median time to onset is 22 months for KS, 32 months for lymphomas, and 69 months for epithelial malignancies [96]. The frequency of KS is higher in liver transplant recipients than it is in other organ transplant recipients [96]. Notably, rare reports of KS in BMT recipients are encountered. KSHV viremia as quantified by a real-time PCR correlates with the progression of KS in transplant recipients [106].

Although the skin is the most commonly involved site of KS, up to 40% of transplant recipients may develop visceral lesions, including gastrointestinal, pulmonary, bladder, and laryngeal KS. Gastrointestinal involvement may often be occult, or it may be associated with nonspecific gastrointestinal symptoms and bleeding. Occasionally, perforation, obstruction, and protein-losing enteropathy caused by lymphatic obstruction may occur. In addition, some authors report nonneoplastic manifestations (e.g., a syndrome characterized by fever, splenomegaly, and marrow failure with plasmacytosis) after transplantation [107].

28.3.3 Management

Reduction or withdrawal of immunosuppression remains the mainstay of the management of KS in transplant recipients. Although the regression of KS with the reduction or cessation of immunosuppression occurs in all forms of KS, remission rates are higher for visceral lesions than for those that are nonvisceral. However, up to 50% of the patients treated with the cessation of immunosuppression may lose their grafts [101, 108]. In patients with disseminated or visceral KS that fails to respond to modifications in immunosuppression, combination chemotherapy has been employed in some reports [109]. Of five transplant recipients treated with combination therapy with doxorubicin, bleomycin, and vincristine, two experienced a complete remission and two had a partial response [109]. In this setting, retransplantation in renal allograft recipients leads to the recurrence of KS lesions almost universally [108]. However, some case studies have reported a successful outcome after retransplantation [110].

The existence of lytically infected cells in KS lesions has implications for the treatment of KS with antiviral agents. Nucleoside analogues (e.g., acyclovir and penciclovir) have minimal in vitro activity against KSHV. Although the virus is susceptible to ganciclovir and foscarnet, the acyclic nucleosides phosphonate analogues, cidofovir and HPMPA, are potent inhibitors of HHV-8 DNA synthesis [111]. Reportedly, adefovir blocks HHV-8 DNA replication at a fourfold lower concentration than does foscarnet [111].

In the setting of HIV, a significant reduction in the risk of KS in patients who receive ganciclovir has been reported; however, experience with antiviral therapy in the transplant setting is limited. In four thoracic organ transplant recipients who received cidofovir for KS recurrence or for intolerance of cytotoxic chemotherapy, researchers documented the clearance of KSHV from the blood in two out of two and from skin lesions in two out of four patients [112]. Clinical improvement of one patient's gastric KS lesions was observed [112]. However, results regarding a role for antiviral therapy in the management of posttransplantation KS are inconclusive.

B cells in the lymph nodes of patients with KSHV-related, multicentric Castleman disease stain positively for CD20 surface antigen; one study used anti-CD20 antibody therapy in a patient with HIV infection [113]. The role of other experimental therapies (e.g., inhibitors of angiogenesis and retinoids) is unproven [114].

The immunosuppressive agent rapamycin has been shown to inhibit the growth of primary effusion lymphoma (PEL) cell lives, and to prevent and delay the development of PEL tumors in animal models of severe combined immunodeficiency [115]. Anecdotal reports have documented regression of KS lesions in transplant recipients upon conversion to rapamycin-based immunosuppression [116, 117]. Others have reported no such beneficial effect [118].

28.4 Summary

Existing literature supports the role of HHV-6 as a potential cause of encephalitis and bone marrow suppression in transplant recipients. An association of HHV-6 with fungal infections and CMV infection has been documented. HHV-7 also appears to be an immunomodulatory agent that may facilitate the pathogenicity of CMV. One possibility is that the beneficial effect of antiviral prophylaxis for CMV in transplant recipients could have been mediated in part through its effect on these newer beta herpesviruses. Trials for CMV chemoprophylaxis may consider assessing the effect of antiviral agents on HHV-6 and HHV-7 since these data have implications for the further elucidation of the pathogenicity of these viruses.

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29
Human Herpesvirus 6A, 6B, 7, and 8 Infections After Hematopoietic Stem Cell Transplantation

Joshua A. Hill and Danielle M. Zerr

29.1 Introduction

Herpesviruses have plagued persons undergoing hematopoietic stem cell transplantation (HCT) since the introduction of this life-saving therapy in the 1950s. With the early recognition of severe disease due to local or disseminated herpes simplex virus (HSV), it was clear that preventative measures were necessary, and acyclovir prophylaxis studies were initiated in the early 1980s [1]. Similarly, human cytomegalovirus (CMV) reactivation was found to occur in the majority of patients, many of whom developed severe pulmonary and gastrointestinal disease unimpeded by acyclovir. This prompted the development of prophylactic and preemptive treatment strategies using ganciclovir and foscarnet [2]. Human herpesvirus (HHV) 6A, HHV-6B, HHV-7, and HHV-8 (also known as Kaposi's sarcoma associated herpesvirus, or KSHV) were unknown in the early era of HCT, only to be discovered in an 8-year period between 1986 and 1994 [3–5].

HHV-6A, HHV-6B, and HHV-7 are T lymphotropic viruses closely related to CMV, placing them in the betaherpesvirus family. Given their etiologic link to roseola infantum, these viruses are designated Roseoloviruses. Population based studies demonstrate that upwards of 95% of the human population is infected in the first few years of life. KSHV is a member of the gammaherpesvirus family in the rhadinovirus genus. Infection with KSHV is less frequent, and seroprevalence ranges widely from <10% to >40% depending on geographic region and risk factors [6–8].

The preponderance of evidence suggests an important role for HHV-6B in complications after allogeneic HCT, whereas there is less support for a significant role for HHV-6A, HHV-7, and KSHV. The utility of screening and preventive approaches for these viruses remains unclear given a still limited understanding of the full spectrum of their clinical impact after HCT, coupled with costly diagnostics and limited low-risk therapeutic options.

This chapter will focus on recent advances in our understanding of the post-HCT epidemiology, clinical impact, diagnosis, and treatment of HHV-6B in particular, in addition to HHV-6A, HHV-7, and KSHV.

29.2 Epidemiology

29.2.1 HHV-6A and B

HHV-6A and HHV-6B were classified as separate species in 2012 due to epidemiological, biological, and immunological distinctions [9]. HHV-6B infects most children within the first few years of life and is likely transmitted by saliva [10, 11]. The epidemiology of HHV-6A is not as well described. Primary infection with HHV-6A appears to occur later in life and with less frequency [12], although early infection may be more common in Sub-Saharan Africa [13].

HHV-6B uses the cellular receptor CD134 for entry into cells [14], whereas HHV-6A uses CD46 [15]. Like other herpesviruses, these pathogens chronically infect their host and remain latent in a variety of cell types, including peripheral blood mononuclear cells (PBMCs), natural killer cells, bone marrow progenitor cells, salivary glands, bronchial glands, oligodendrocytes, and astrocytes [10, 16, 17]. Although the putative method of latency remains unclear, there is mounting evidence to support subtelomeric chromosomal integration rather than episome formation, a mechanism unique to HHV-6 species among HHVs [18, 19] (Figure 29-1). If viral integration occurs in a germ-line cell, vertical transmission of chromosomally integrated HHV-6 (ciHHV-6) results in offspring with latent HHV-6 in all nucleated cells. This condition of inherited ciHHV-6 is found in ~1% of the population (~70 million individuals worldwide). HHV-6B is responsible for two-thirds of inherited ciHHV-6 cases while HHV-6A accounts for one-third [18]. Importantly, viral integration is not a dead end, as reactivation with associated cytopathic effects can be induced in vitro [20], and a case of apparent inherited ciHHV-6-associated disease was recently described in a child with severe combined immunodeficiency who underwent HCT [21].

HHV-6B accounts for ~98% of HHV-6 species reactivation after allogeneic HCT [22–26]. Studies of HHV-6B detection in diverse populations of allogeneic HCT recipients, primarily using polymerase chain reaction (PCR) assays to detect viral DNA, demonstrate that 40–50% of

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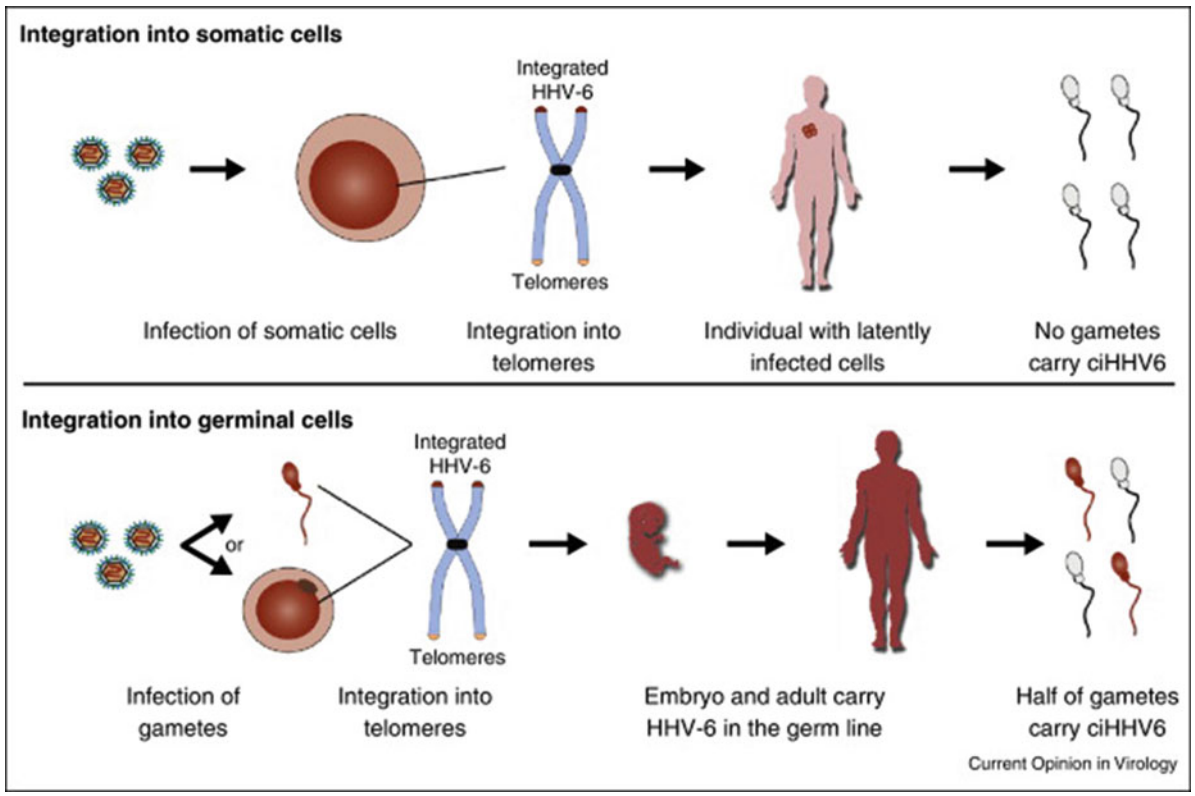

FIGURE 29-1. Chromosomal integration of HHV-6A and B. Human herpesvirus 6A and 6B are able to integrate into the subtelomeric and/or telomeric region of human chromosomes. *Upper panel*: This demonstrates a potential pathway of latency in a naturally infected individual in whom the virus integrates into a chromosome of infected cells. *Lower panel*: This demonstrates the unique condition of inherited chromosomally integrated HHV-6 that occurs when the virus infects and integrates into the chromosome of a gamete that is subsequently fertilized. This will result in an individual with one copy of the entire HHV-6 genome in every nucleated cell and in 50% of their gametes, allowing for vertical transmission. Reprinted from Current Opinion in Virology, Volume 9, Kauffer BB and Flamand L, Chromosomally integrated HHV-6: impact on virus, cell and organismal biology, 111–118, Copyright 2014, with permission from Elsevier.

TABLE 29-1. Epidemiology of HHV-6A, HHV-6B, HHV-7, and KSHV infections in HCT recipients

	Population-based seroprevalence	Incidence of reactivation (%)	Risk factors for reactivation after HCT
HHV-6A	Not well studied	0–3[a]	Not well understood
HHV-6B	~95% after infancy	40–50	Cord blood HCT HLA mismatched donor Unrelated donor Acute graft-versus-host disease grades II–IV Receipt of anti-T-cell antibodies Receipt of steroids
HHV-7	~95% after infancy	20–60	Not well understood
KSHV	Sub-Saharan Africa, >30–40% Mediterranean, 10–20% Americas and Asia, <10% Men who have sex with men, 20–40%	0–1	Not well understood

[a]Consider latent inherited ciHHV-6A as a source of viral DNA.

patients have viral reactivation at a median of approximately 3 weeks after HCT (Table 29-1). Given that the majority of people are seropositive for HHV-6B at the time of HCT, most viral detection is likely due to viral reactivation; however, as a number of leukocyte subsets are sites of latency for HHV-6 [16, 17], there is also the possibility of graft-induced (re-) infection with HHV-6 from donor to recipient. The most common risk factors for HHV-6 reactivation include cord blood transplantation (CBT), use of a mismatched or unrelated donor, receipt of anti-T-cell antibodies, acute

graft-versus-host disease (GVHD) grades II–IV, and treatment with glucocorticoids [23, 24, 27–32] (Table 29-1). The highest rates of HHV-6B detection are seen after CBT; in a recent prospective cohort of 125 CBT recipients, reactivation was documented in 94% of patients [25]. CBT recipients also have higher levels of HHV-6 reactivation with median viral loads approximately 1 $\log_{10}$ higher than recipients of other sources of stem cells (i.e., PBMCs or bone marrow) [25, 33]. While use of alternative donor sources and graft manipulation, such as cord blood cells and T-cell depletion, has allowed for expanded access to HCT, later engraftment and delayed adaptive immune reconstitution have resulted in a greater burden of virus-associated complications [34]. A recent study also demonstrated that up to 50% of CBT recipients have long-lasting detection of HHV-6B a median of 4 years post-CBT, despite no significant differences in long-term immune reconstitution [35].

29.2.2 HHV-7

HHV-7, similar to HHV-6B, infects most children within the first few years of life and is thought to be transmitted via saliva [3, 10, 36, 37]. HHV-7 exhibits selective tropism for CD4+ T-cells and uses the CD4 receptor for cell entry [38]. Primary infection rarely comes to clinical attention but is likely another, although less frequent, cause of roseola infantum. Most studies of HHV-7 after HCT have been conducted in pediatric patients, who have a 20–60% incidence of HHV-7 DNA detection, typically during the first 2–6 weeks after HCT [39–41] (Table 29-1). One study reported a lower rate of detection (5.5% of patients) and detection in allogeneic but not autologous HCT recipients [42]. In contrast to HHV-6B, there is no obvious peak time of reactivation, and the vast majority of reactivation events are transient and low-level. Risk factors for HHV-7 reactivation have not been well studied. A prospective study assessing for HHV-7 reactivation with weekly PCR testing for 12 weeks after autologous and allogeneic HCT in 59 pediatric patients showed a negative association with HHV-6 reactivation and an increased incidence after autologous (vs allogeneic) HCT and PBMC (vs cord blood/bone marrow) HCT in univariate models [39]. Another study in 125 pediatric HCT recipients reported a positive association with higher dose of CD34+ stem cells, use of busulfan, related donor, and HHV-6 reactivation.

29.2.3 KSHV (or HHV-8)

KSHV infection is less ubiquitous than HHV-6A, HHV-6B, and HHV-7. Although the timing and precise route of infection are not well understood, exposure to saliva through casual contact among children and young adults is the most plausible route of infection in the majority of individuals [43]. CD19+ B cells appear to be the primary viral reservoir, but other potential reservoirs, such as the endothelium, CD68+ monocyte-macrophage cells, salivary glands, and prostate epithelia have been proposed [6]. There are notable geographic differences in the seroprevalence of KSHV, with the highest rates in sub-Saharan Africa (>30–40%), intermediate rates in Mediterranean areas (10–20%), and lowest rates in Asia and the USA (<10%) [6] (Table 29-1). KSHV infection is evident in 20–40% of men who have sex with men [6–8], and sexual transmission has been demonstrated within this population [44]. Interestingly, KSHV is not detected in appreciable amounts in seminal fluid or the rectum [45].

An epidemiologic study of 187 HCT donor–recipient pairs in Italy found that 13% of donors and 11% of recipients were seropositive for KSHV [46]. KSHV DNA detection in the blood was rare (0–1%) after HCT in this and other studies [47] (Table 29-1). Among solid organ transplant recipients developing KS, more than 80% appear to be seropositive prior to transplant, suggesting viral reactivation as a cause of disease [48]. The natural history of KSHV after HCT is poorly studied and may be higher in certain patient populations.

29.3 Clinical Impact

29.3.1 HHV-6A and B

HHV-6B is a pleiotropic virus that has been associated with many complications after allogeneic HCT (Table 29-2). Whether HHV-6B has a causal role in all of these processes has been controversial due to the challenge implicit in

Table 29-2. Disease associations with HHV-6A, HHV-6B, HHV-7, and KSHV reactivation after HCT

	Epidemiologic associations	Level of in vitro or in vivo support for causation
HHV-6A	Encephalitis[a]	Weak, case reports
HHV-6B	Encephalitis (including limbic encephalitis)	Strong
	CNS dysfunction (not encephalitis)	Moderate
	Fever and rash	Strong
	Myelosuppression	Strong
	Acute graft-versus-host disease	Moderate
	Allograft rejection	Weak
	CMV reactivation	Weak
	Pneumonitis	Weak
	Hepatitis	Weak, case reports
	Increased all-cause mortality	Weak
HHV-7	Encephalomyelitis	Weak, case reports
	Increased level and duration of CMV viremia	Weak
KSHV	Kaposi's sarcoma	Strong
	Bone marrow failure	Weak
	Fever and rash	Weak

[a]Consider latent ciHHV-6A as a source of viral DNA.

attributing disease to a virus latent in diverse cell types. Studies have used a variety of techniques without international standards on an array of sample sources to detect HHV-6, often without distinguishing between species, making direct comparisons problematic. HHV-6A has not been independently associated with any disease after HCT.

29.3.1.1 Central Nervous System Disease

There is sufficient evidence to implicate HHV-6B as a cause of encephalitis, and the most common etiology of encephalitis after HCT, as recently reviewed by Drs. Ward and Ogata [49, 50]. The incidence of HHV-6B encephalitis after allogeneic HCT was evaluated in a meta-analysis of 19 studies and reported to occur in 8.3% of CBT recipients compared to 0.5% of patients receiving stem cells derived from PBMCs or bone marrow [51].

Many studies have defined HHV-6 encephalitis as acute encephalopathy with HHV-6 detected in the CSF or brain, without a more likely explanation identified after extensive evaluation. The more specific syndrome of HHV-6B post-HCT acute limbic encephalitis (HHV-6B-PALE) is characterized by distinct clinical, laboratory, and radiographic features, including amnesia (especially anterograde), seizures, confusion, the syndrome of inappropriate antidiuretic hormone secretion, mild cerebrospinal fluid (CSF) pleocytosis and protein elevation, and medial temporal lobe changes on brain magnetic resonance imaging (MRI) [22, 24, 50–52].

Detection of HHV-6B DNA in CSF is a defining clinical criteria for HHV-6B encephalitis, and at least 85% of affected patients have concurrent detection in the blood [24, 51]. In a review of 48 published cases and case series of patients with likely HHV-6B encephalitis over a 15-year period, 40 of 48 cases had HHV-6B documented in CSF samples [53]. All cases followed allogeneic HCT except for 1 after autologous HCT. Symptom onset occurred after engraftment in the majority of cases following adult HCT but may occur pre-engraftment after CBT [24]. The most common neurologic symptoms were confusion and depressed consciousness beginning at a median of 24 days after HCT, with a range of 15–60 days. Seizures were reported in 40% of cases, although electrographic seizures without obvious clinical findings appeared to occur in a higher proportion. MRI findings were abnormal in 30 of 43 cases and involved the medial temporal lobes in 22 of 30 cases. Typical MRI abnormalities include well circumscribed, hyperintense, nonenhancing lesions involving the medial temporal lobes, and especially the hippocampus, on T2, fluid attenuation inversion recovery (FLAIR) and diffusion-weighted (DWI) MRI sequences [52, 54, 55]. Brain computed tomography scans are typically normal. CSF findings were significant for elevated protein levels in 58% of reported cases, as well as mild lymphocytic pleocytosis in a minority of patients (although leukopenia was likely present at the time of lumbar puncture in many cases) [53]. HHV-6B was identified in 24 of 27 cases that underwent discriminatory testing for HHV-6 species. Additional studies have demonstrated HHV-6B protein expression in the mesial temporal lobes of patients with HHV-6B encephalitis [56, 57].

HHV-6 systemic reactivation with high levels of viremia is the primary risk factor implicated in the development of HHV-6B encephalitis. Patients with HHV-6B encephalitis typically have plasma viral loads 100-fold greater than other viremic patients [29, 58]. Cord blood HCT recipients are at particularly high risk for increased frequency and degree of HHV-6 systemic reactivation [25, 33] with subsequent encephalitis [24, 59]. Among patients with viremia, viral loads $>10^4$ copies/ml are typically seen in the setting of HHV-6B encephalitis. A large retrospective study demonstrated that peak plasma HHV-6B viral loads $\geq 10^5$ copies/ml was 71% sensitive and 94% specific for HHV-6B encephalitis [24]. However, it is important to note that some cases of encephalitis have been reported in the absence of viremia, although this may have been do to lack of or the timing of testing. Finally, not all studies tested patients for inherited ciHHV-6, and inappropriate inclusion of affected patients may have led to a relative increase in observed HHV-6 viral loads (see Sect. 29.3.1.3).

Patients who develop HHV-6B encephalitis have significant morbidity and mortality despite antiviral treatment [60]. Retrospective reviews demonstrate that clinical status improves after antiviral treatment in the majority of patients, but 20–40% of patients have persistent mild-to-moderate neurocognitive deficits and up to 25% have progressive encephalitis resulting in death [52, 53, 61]. Mortality is particularly high after CBT with rates of up to 50% [24].

In addition to overt encephalitis, there is accumulating evidence that HHV-6B may also contribute to less-fulminant CNS dysfunction. A large prospective study of 315 allogeneic HCT recipients that systematically assessed for CNS dysfunction found an independent and temporal association between HHV-6B reactivation and both delirium and neurocognitive decline after HCT [62]. Similar findings were demonstrated in a cohort of 35 CBT recipients [63]. HHV-6B DNA detection in CSF is typically associated with neurologic findings ranging from headache to fulminant encephalitis, but it may also occur in the absence of CNS symptoms in a minority of patients [64–66]. Our understanding of the significance of HHV-6B DNA detection in the CSF continues to evolve but is limited by the lack of routine CSF sampling in asymptomatic HCT recipients early after HCT.

29.3.1.2 Non-Central Nervous System Disease

HHV-6B reactivation has been associated with a number of non-CNS conditions after HCT (Table 29-2), although its precise role in these disorders remains unclear. Of all the non-CNS disease associations, HHV-6B as a cause of

myelosuppression has the most supporting evidence; however, intriguing evidence exists for other outcomes as well.

A number of studies have demonstrated an association of HHV-6B reactivation with myelosuppression and delayed engraftment, particularly involving platelets, in addition to graft failure [30, 32, 67–69]. A recent large prospective trial in 235 consecutive allogeneic HCT recipients showed delayed platelet engraftment among the 48% of patients who developed HHV-6B viremia within 100 days [26]. A retrospective study of 77 CBT recipients found a strong association between HHV-6B reactivation and graft failure in adjusted analyses [70]. In vitro studies have provided plausible mechanistic explanations for these findings, including suppression of thrombopoietin-inducible megakaryocytic colony formation [71] and attenuated lymphocyte proliferation responses in the setting of lytic HHV-6B infection [72]. Interestingly, in the acute care setting, HHV-6 primary infection has been associated with relatively lower platelet counts than that found with other childhood causes of fever [73].

Acute GVHD is another important post-HCT complication that has been associated with HHV-6B reactivation [24, 74, 75]. In a prospective trial of 315 allogeneic HCT recipients with twice-weekly PCR testing, HHV-6B was independently and quantitatively associated with acute GVHD grades II–IV in time-dependent multivariable analyses, and the findings suggested a potential role in each of the organ-specific subtypes [23]. Interestingly, a retrospective study found a bidirectional association between HHV-6 reactivation and acute GVHD [76]. This study also showed that HHV-6B reactivation was associated with rash that clinically imitated skin GVHD in 17 patients; histopathology revealed a lymphoid infiltrate but no evidence of acute GVHD. A causal relationship between HHV-6B reactivation and acute GVHD is supported by in vitro and limited clinical data demonstrating that HHV-6 infection may cause a proinflammatory cytokine response (primarily elevated IL-6 concentrations) [58, 77] or type I immune response [78] that may be involved in the development of acute GVHD. Given the morbidity and mortality associated with acute GVHD, additional study of the causal pathways in the development of HHV-6B-associated acute GVHD is warranted.

HHV-6B reactivation has been variably associated with an increased risk for CMV reactivation and disease after HCT. A recent prospective study of 315 allogeneic HCT patients demonstrated that HHV-6B reactivation was quantitatively associated with increased risk of subsequent CMV reactivation [23]. In a study of 21 allogeneic HCT recipients, HHV-6 reactivation was associated with an absence of CMV-specific lymphocyte proliferative responses, and persistence of HHV-6 detection correlated with need for repeated courses of preemptive antiviral therapy against CMV during the first 6 months after HCT [79]. In contrast, a study of 68 allogeneic HCT recipients found that HHV-6 reactivation was associated with CMV reactivation in univariate analysis but not multivariable analyses [80]. This report additionally demonstrated that detection of HHV-6 DNA in plasma did not seem to affect CMV-specific T cell immune reconstitution as measured by intracellular cytokine staining. The biologic plausibility of a causal link between HHV-6B and subsequent CMV reactivation is supported by in vitro studies demonstrating the immunosuppressive effects of HHV-6B reactivation, potentially through inhibition of IL-12 production [81, 82]. In addition, the generalizability of the association between HHV-6 and CMV reactivation is supported by results from clinical studies of non-HCT populations including critically ill adults and solid organ transplant recipients [83–85].

HHV-6B has been implicated with other end-organ diseases after allogeneic HCT in case reports and small case series. Perhaps the most important area of investigation is its role in pulmonary disease, which remains a leading cause of morbidity and mortality after HCT and is idiopathic in ~10% of patients [86–90]. A number of studies have reported a potential association between HHV-6 and pneumonitis, as first described in two allogeneic HCT recipients with pneumonitis and HHV-6 detection in respiratory specimens and lung tissue by PCR and immunohistochemistry [91]. A retrospective study of lung biopsy specimens from 15 allogeneic HCT recipients with pneumonia found a quantitative association between HHV-6 detection and idiopathic pneumonia syndrome [92]. Several subsequent retrospective studies have had variable results, and HHV-6 has been frequently detected along with copathogens and in control subjects [93–95], raising questions about whether the association is truly causal. In a recent study employing quantitative PCR testing for known and potential pulmonary pathogens in 69 allogeneic HCT recipients with idiopathic pneumonia syndrome (IPS), HHV-6 was detected in 29% of patients, and it was the only pathogen found in half of those patients [96]. Mortality was significantly higher in patients with IPS and HHV-6 detection compared to those without a pathogen, and blood contamination did not appear to be a confounder. Past studies have been limited by small sample size and lack of speciation and quantitative diagnostics with an international standard. Additional study of HHV-6 and lung disease is warranted.

There are limited data suggesting that HHV-6 may be a pathogen in liver disease after HCT. HHV-6 infection and replication in human liver cells has been demonstrated in vitro [97, 98], and multiple studies have implicated HHV-6 as a cause of hepatitis in immunocompetent individuals [99–101]. Three cases of HHV-6-associated hepatitis after allogeneic HCT have been described in patients with hepatitis and temporally associated HHV-6 detection in blood and/or liver tissue, along with histopathological and/or immunohistochemical evidence of active viral replication in the liver [30, 102, 103]. One case was documented to be due to HHV-6B. All three cases improved with antiviral therapy. Given the high incidence of HHV-6B reactivation after HCT and lack of routine screening, HHV-6B may be an underappreciated cause of liver disease after HCT.

No disease has been causally linked with HHV-6A, which is infrequently described in immunocompromised patients and only accounts for 0–3% of reactivation events after HCT [23, 104]. There are a few case reports of HHV-6A encephalitis after HCT [105–107]. With a contemporary understanding of HHV-6 biology, these reactivation events and cases of encephalitis were most likely due to unrecognized inherited ciHHV-6A. Whether HHV-6A was contributory to the patients' illness in those with encephalitis is hard to know with the information provided [50]. However, two recent case reports of patients with inherited ciHHV-6A were highly suggestive of HHV-6A reactivation from the integrated virus with associated encephalitis [108] and hemophagocytic lymphohistiocytosis [21]. Ultimately, HHV-6A detection in the setting of HCT is suggestive of inherited ciHHV-6A, and it appears that viral reactivation from this integrated state can occur, as discussed further in the next section. However, due to the low incidence of HHV-6A reactivation after HCT, it remains unclear if it shares an association with the diversity of complications seen in the context of HHV-6B reactivation.

29.3.1.3 Inherited Chromosomally Integrated HHV-6

Inherited ciHHV-6 is present in approximately 1–2% of the general population (Figure 29-1). Lack of recognition of this condition has led to inappropriate treatment of patients and considerable confusion in the literature, when detection of viral DNA did not correlate with clinical disease [109, 110]. Whether patients with inherited ciHHV-6 can develop HHV-6 reactivation with associated pathogenicity, or other indirect complications, has been a topic of controversy. There is a dearth of studies exploring clinical outcomes in affected individuals. However, an accumulating body of in vitro and in vivo evidence suggests that inherited ciHHV-6 can be the source of pathogenic viral reactivation and other complications, especially in immunocompromised patients.

The prevalence of inherited ciHHV-6 among hospitalized patients was >3-fold higher than in healthy blood donors in one report, suggesting that persons with inherited ciHHV-6 may have more medical complications [111]. In a study of 37 patients with a diagnosis of HHV-6 CNS dysfunction after HCT, there was no obvious enrichment for inherited ciHHV-6 to suggest that patients with this condition have greater risk for disease due to HHV-6 [108]. However, an estimate of the true prevalence was limited by the sample size. A recent report detailed in vivo molecular and virological evidence of HHV-6A reactivation from inherited ciHHV-6A, with associated clinical symptoms of hemophagocytic lymphohistiocytosis, in a child with X-linked severe combined immunodeficiency who underwent HCT [21]. A few case reports and small studies also suggest that inherited ciHHV-6 may directly or indirectly contribute to clinical disease in affected patients [108, 112–114]. However, a retrospective review of 21 cases of patients who had or received stem cells or organs with inherited ciHHV-6 found no clinical disease associations [115].

In vitro experiments have provided clear evidence of HHV-6 transcription and translation from ciHHV-6 cell lines. Researchers have successfully induced lytic genes and capsid mRNA expression by exposing ciHHV-6 cell lines to HDAC inhibitors [20, 116]. Furthermore, these cell lines were demonstrated to give rise to propagating virus with cytopathic effects in an exposed naïve T-cell line, providing evidence of viral fitness. A recent study of 11 immunocompetent and immunocompromised patients with inherited ciHHV-6 used RT-PCR to demonstrate evidence of active viral transcription in 4/11 and HHV-6 antigen detection in 7/10 individuals, although results were not correlated with clinical symptoms [117]. Given the relatively high frequency of inherited ciHHV-6 in the population, it is critical that larger studies evaluate the clinical significance of this condition. Experts have suggested caution in using specimens with inherited ciHHV-6 for human stem cell or organ transplantation [118, 119]. There is an urgent need to address if, how, and when inherited ciHHV-6 may have deleterious effects in its host.

29.3.2 HHV-7

The significance of HHV-7 DNA detection after HCT remains unclear, although the virus is rarely associated with any clinical manifestations (Table 29-2). Some studies suggest that patients with HHV-7 reactivation have higher CMV viral loads or longer duration of CMV detection [40, 120], and a few case reports have implicated HHV-7 as a cause of encephalomyelitis after allogeneic HCT [121–123]. Most studies of HHV-7 in immunocompromised patients have had small sample sizes, often with conflicting results.

29.3.3 KSHV (or HHV-8)

As in other human HHV-associated diseases, KSHV primarily causes disease in immunocompromised subjects due to reactivation of latent infection or transmission of infected cells from the donor. KSHV infection is the cause of Kaposi's sarcoma (KS), which has four clinical presentations: classic, endemic, epidemic or acquired immune deficiency syndrome (AIDS)-associated, and immunosuppression or transplant-associated [7]. In each case, the degree of immunosuppression is a primary factor in the development and progression of disease. While post-HCT KS is rare, there are about 20 reported cases in the literature described primarily after allogeneic HCT [124–127]. In the HCT setting, KS is usually associated with skin lesions and bone marrow failure [124]. A review of 14 cases reports a median age of 46 years (range 7–69), male:female ratio of 2.5:1, and median interval from HCT to KS diagnosis of 8.5 months (range 3–27) [126].

The skin was involved in the majority of patients, and 50% had diffuse disease involving >1 site or multiple organs. Overall mortality was around 35%, although deaths occurred exclusively in patients with diffuse disease, indicating a 70% mortality rate among this patient group.

Clinical manifestations associated with any KSHV reactivation or infection after HCT have been infrequently reported and include fever, rash, hepatitis and bone marrow failure [128, 129] (Table 29-2). Most studies have not found an association between KSHV seropositivity or post-HCT seroconversion and the development of clinical symptoms or increased mortality [46].

29.4 Diagnosis

29.4.1 HHV-6A and B

Diagnosis of clinically relevant HHV-6 species infection or reactivation is challenging due to the high prevalence of primary infection with persistence of the virus in a myriad of cell types [11, 12]. A variety of techniques can be used to test for HHV-6, including serological studies, antigen detection, isolation by culture, and nucleic acid detection. Due to the labor-intensive nature of many of these approaches, in addition to lack of specificity and/or inability to distinguish between HHV-6A and HHV-6B, quantitative viral nucleic acid detection by PCR has become the method of choice. Advantages of this method include its speed, sensitivity, specificity, and ability to distinguish and quantitate species A and B [130–133].

Many studies have demonstrated that detection of HHV-6 DNA in plasma, serum, or CSF correlates well with active viral replication [53, 57, 134]. One study found the specificity of HHV-6 DNA detection in plasma by quantitative PCR to be 84% based on a comparison with viral culture [135]. However, there are a number of important limitations to consider when interpreting results from PCR detection of HHV-6 DNA. *First*, detection of HHV-6 DNA in liquid compartments (e.g., blood, CSF, bronchoalveolar lavage fluid) may significantly underestimate tissue-level viral replication. For example, brain tissue from patients with HHV-6 encephalitis has been shown to have higher levels and prolonged detection of HHV-6 DNA compared to blood or CSF samples [56, 57]. *Second*, sample selection and processing for HHV-6 PCR testing can markedly affect results. For instance, detection of HHV-6B DNA in whole blood or PBMCs does not correlate as well with active viral replication, as the mononuclear cell is a site of latency [17]. The use of serum or plasma is thought to be more representative of cell-free replicating virus, with the caveat that viral DNA may originate from latently infected cells that have lysed during sample preparation [136]. *Third*, the lack of an international standard for HHV-6A and B DNA measurement precludes extrapolation of quantitative levels to other studies, as interlaboratory correlation is known to be poor [131, 137]. The development of an international standard, such as the one for CMV recently made available by the World Health Organization [138], is currently underway. Whether routine screening in all or select HCT recipients will improve outcomes needs further study, and the establishment of viral load thresholds to guide intervention will continue to be limited until standardization in testing assays, sample selection, and sample processing has been accomplished.

The condition of inherited ciHHV-6 adds particular challenges to HHV-6 diagnostics and interpretation of results. Inherited ciHHV-6 is suggested by HHV-6 DNA levels of >5.5 $\log_{10}$ copies/ml in whole blood specimens, corresponding to one copy of HHV-6 per nucleated cell in the sample [18] (Figure 29-1). Other findings consistent with inherited ciHHV-6 include persistent viral DNA detection without a decrease in quantitative levels despite appropriate antiviral treatment. This can be true even when plasma or serum is used due to release of latent viral DNA from cell lysis or contamination, especially if there is a delay in sample preparation and testing [139]. The gold standard for diagnosis of inherited ciHHV-6 is fluorescence in situ hybridization, a labor-intensive procedure with limited availability [139]. Another approach has been to test hair follicles, where any detection of HHV-6 is consistent with inherited ciHHV-6; however, most laboratories are not equipped to test such samples. Droplet digital PCR (ddPCR) is an emerging technique that allows for absolute and precise quantitation of target DNA without the use of a standard curve [140, 141]. This method is particularly well suited for the identification of inherited ciHHV-6 and has recently been developed for this purpose [142, 143]. By concurrently amplifying DNA targets for HHV-6 and a reference human gene for cell count (e.g., human ribonuclease P [RPP30]), a ratio of HHV-6 DNA to cell genome equivalents of ~1 is indicative of inherited ciHHV-6. These assays have high sensitivity and specificity when used with PBMCs and other highly cellular samples, but they can also be utilized with archived plasma, sera, and other samples to aid in retrospective research, although with reduced specificity [142].

Future directions for the diagnosis of active HHV-6 infection include detection of RNA via reverse transcription real-time quantitative PCR (RT-qPCR) [130, 144, 145]. This method of amplifying messenger RNA from PBMCs or other specimens could provide a better approach to distinguish active from latent infections [144], and it may be particularly useful for identifying HHV-6 reactivation in patients with inherited ciHHV-6. One study comparing viral culture with a nested RT-PCR assay for a late protein (*U100*) demonstrated 95% sensitivity and 98.8% specificity of the RT-PCR assay for actively replicating virus in PBMC samples [146]. Additionally, in-depth molecular interrogation for evidence of HHV-6 infection and replication in tissues and other samples involved in end-organ disease is required to identify pathologic and molecular signatures of HHV-6B infection [147].

While the evidence does not yet support routine monitoring for HHV-6B after HCT, patients with acute encephalopathy should have testing of plasma and CSF for HHV-6B. HHV-6B testing may also be considered for patients with end-organ disease that has previously been associated with HHV-6B, especially when no other explanation exists. There is also no basis for routine screening of donors and recipients for inherited ciHHV-6 at this time, although focused testing is important in individuals with findings consistent with ciHHV-6. The implementation of novel diagnostic approaches in well-designed and appropriately powered studies is an essential next step for establishing causality and moving the field forward from studies of association to studies of causation. Large, standardized studies to evaluate the correlation between HHV-6 RNA and DNA detection in blood and tissue with associated end-organ disease will be critical to establish actionable indicators for treatment.

29.4.2 HHV-7 and KSHV (or HHV-8)

As discussed for HHV-6 species, the mainstay for detection of HHV-7 and KSHV in the clinical setting is real time quantitative PCR. There are also no international standards for PCR tests for these viruses. In patients with KS, KSHV viral load in PBMCs correlates with tumor burden, although data in the HCT setting is lacking [48, 148]. Although KSHV viremia is associated with KS and disease progression, diagnosis is predicated on tissue biopsy, which reveals pathognomonic findings. Typical histopathologic features include ectatic, irregularly shaped, round capillary, and slit-like endothelium-lined vascular spaces and spindle-shaped cells accompanied by a variable inflammatory mononuclear cell infiltrate. Kaposi sarcoma cells stain for endothelial cell markers such as $CD34^+$, and immunostaining of anti-HHV-8 antibodies may be useful for diagnosis of early stage disease. Due to the unclear significance of HHV-7 detection in blood specimens after HCT and the low incidence of KSHV-associated disease in this setting, routine screening for these viruses is not currently recommended.

29.5 Treatment and Prevention

29.5.1 HHV-6A and B

Optimal treatment approaches (i.e., single or combination antivirals, dose, duration) have not been rigorously studied. Because of this, there are no widely adopted standard practice guidelines for HHV-6A and B except for treatment of post-HCT HHV-6B encephalitis [149–151]. However, several available antiviral agents demonstrate good in vitro and suggestive in vivo activity against HHV-6A and B, including foscarnet, ganciclovir, and cidofovir [12, 105, 152–154]. Based on these data, treatment recommendations for HHV-6B encephalitis include the use of ganciclovir and/or foscarnet, either alone or in combination [149–151]. Although treatment practices vary, one approach is to use foscarnet (90 mg/kg twice a day) pre-engraftment and ganciclovir (5 mg/kg twice a day) post-engraftment for at least 3 weeks and until clearance of HHV-6 DNA in plasma and CSF by PCR testing (expert opinion).

Prophylactic and preemptive strategies to mitigate HHV-6B reactivation, such as those employed for other HHVs (e.g., HSV, VZV, CMV), have been reported. The use of prophylactic ganciclovir in HCT patients can reduce HHV-6B reactivation in the blood [84] and may reduce associated morbidity in high-risk patients [155, 156]. Other studies have not found an association between reduction in incidence and level of HHV-6B detection and outcomes [157, 158]. Large-scale adoption of this approach is limited by the risk of ganciclovir-induced myelosuppression given the relatively low incidence of serious HHV-6-associated end-organ disease and a limited understanding of the significance of HHV-6B reactivation in the absence of apparent disease. In a retrospective study evaluating the utility of low dose foscarnet (50 mg/kg/day) for 10 days post-engraftment to prevent HHV-6B reactivation in a cohort of 118 allogeneic HCT recipients (unrelated or cord blood donors), high-level HHV-6B reactivation (>10,000 copies/ml) and encephalitis were reduced but did not reach clinical significance [159]. Two small prospective studies of preemptive ganciclovir (5–10 mg/kg/day) or foscarnet (90 mg/kg/day) and one prophylactic trial with foscarnet (90 mg/kg/day) after HCT did not significantly reduce the complications of HHV-6B reactivation [158, 160]. This is perhaps due to the dynamic kinetics of plasma HHV-6 DNA detection and potential delay in time to plasma detection after tissue-level reactivation.

Drug-resistant strains of HHV-6 appear to be exceedingly rare; while in vitro studies support the potential for HHV-6 to develop resistance to antiviral agents, only a few case reports have described the emergence of drug-resistant isolates in the clinical setting [161–164]. However, because the currently available antiviral medications have significant toxicities and all act via similar mechanisms to antagonize DNA polymerase activity, the development of novel therapies is a priority.

A number of new treatment modalities that have activity against HHV-6 species are in various stages of development and testing. Brincidofovir, or CMX-001, is a lipophilic derivative of cidofovir that has generated significant excitement in the field of HCT due to its broad range of activity against many DNA viruses, oral administration, favorable side-effect profile, and high in vitro activity with half maximal effective concentration (EC50) values of 3 and 7 nM for HHV-6A and B, respectively [165]. While this and other available anti-HHV-6 drugs target the viral DNA polymerase, CMV423 is

a novel antiviral agent with potent in vitro activity against the beta herpesviruses through inhibition of a cellular tyrosine kinase involved in viral replication [166]. It has compared favorably to ganciclovir and foscarnet due to its high activity and low cytotoxicity. A variety of other molecules have been reported to exhibit antiviral activity against HHV-6 in cell culture but are in early stages of development [154], and none of these novel agents have been well studied for use against HHV-6 species in the clinical setting. The production of HHV-6 specific cytotoxic T cells for adoptive immunotherapy has been encouraging but is in early stages of development [167–169].

Further testing and discovery of therapies with low toxicity and high efficacy against HHV-6 species is imperative for the implementation of clinical trials exploring the impact of prophylactic, preemptive, and targeted treatment strategies. Prophylactic approaches may be important, as viral detection in liquid compartments such as blood may underestimate tissue-level reactivation. Continued refinement of our understanding of risk factors for HHV-6 reactivation, replication kinetics, and clinical impact may allow for focused treatment of high-risk patients using newly developed agents.

29.5.2 HHV-7

Treatment for HHV-7 has been less well studied given its unclear clinical significance after HCT and in other settings. Like HHV-6 species, acyclovir and other thymidine kinase-dependent drugs are only marginally effective in vitro, while ganciclovir, foscarnet, and cidofovir show similarly potent inhibition of HHV-7 replication in vitro and possibly in vivo [12, 170, 171].

29.5.3 KSHV (or HHV-8)

Treatment of KSHV after HCT is predicated on reduction of immunosuppression, particularly in the setting of localized disease [48, 126]. Chemotherapeutic treatments, such as liposomal anthracyclines (e.g., doxorubicin) and taxanes (e.g., docetaxel), or radiotherapy, might be considered in the setting of diffuse or refractory disease, but the toxicities after HCT should be carefully considered. Sirolimus appears to have antineoplastic activity through its inhibition of the mammalian target of rapamycin (mTOR) and angiogenesis, and it has been reported to contribute to KS regression in one post-HCT patient [8]. Although in vitro studies have demonstrated anti-KSHV activity for ganciclovir, foscarnet, and cidofovir, clinical efficacy in patients with KS has not been demonstrated [48]. This is likely because most tumor cells in KS are latently infected with KSHV, and the anti-DNA polymerase mechanism of these drugs requires actively replicating virus for efficacy.

29.6 Conclusion

- Establishing HHV-6A, HHV-6B, and HHV-7 as causal pathogens in associated diseases is challenging due to their ubiquitous infection of the human population with latency in diverse cell types.
- The importance of HHV-6B reactivation after HCT is underscored by its association with encephalitis and mortality in this setting [23, 26, 172].
- The clinical role of HHV-7 after HCT remains unclear.
- While the incidence of KS due to KSHV after HCT is low, affected patients may suffer significant morbidity, and our ability to predict or prevent development of this disease is poorly understood.
- Actionable interpretation of prior and ongoing studies of these viruses continues to be constrained by existing diagnostic methods and study designs which lead to inconclusive results. Future investigation of both direct and indirect mechanisms by which these viruses contribute to disease, using improved characterization of viral, host, and clinical factors associated with their pathogenesis, will be critical to advance our understanding of their clinical significance.
- As new antiviral therapies emerge [167, 173], the ability to define endpoints and risk-stratify patients is increasingly important. Targeted screening, coupled with low risk prevention and treatment strategies, may improve patient outcomes after HCT. Multicenter randomized controlled treatment and prevention trials using agents active against these viruses are greatly needed to explore a causal link between viral reactivation and adverse outcomes in HCT recipients.

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30 Influenza and Parainfluenza Infection in Hematopoietic Stem Cell and Solid Organ Transplant Recipients

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30.1 Influenza Infection in Hematopoietic Stem Cell Transplant and Solid Organ Transplant Recipients

30.1.1 Overview and Epidemiology

The influenza virus is among the most common human respiratory viruses. It belongs to the *Orthomyxoviridae* family and is a segmented, single-stranded RNA virus in which the individual segments code for critical peptides. Further classification of the virus into subtypes is based on its surface hemagglutinins and neuraminidases [1]. Seasonal influenza activity can begin as early as October and continue into May in the Northern hemisphere. In the USA, influenza activity most commonly peaks between December and February [1]. During the 2014–2015 season, the Centers for Disease Control and Prevention (CDC) reported 125,462 infections (18.1% of specimens tested) (Figure 30-1). The proportion of deaths during this time period attributed to pneumonia secondary to influenza according to the 122 Cities Mortality Reporting System, ranged from 5.0% to 9.3% [2].

Seasonal influenza virus infections in humans cause annual epidemics resulting in millions of cases worldwide and significant health and economic burdens [3]. The seasonal prevalence of influenza infections in immunocompromised patients, including solid organ transplant (SOT) and hematopoietic stem cell transplant (HCT) recipients, closely parallels the community-wide prevalence (Figure 30-1), as demonstrated during local epidemics and the 2009 H1N1 pandemic [1, 3, 4]. Although the overall incidence is low (<5%) [5, 6], influenza infection remains a significant cause of morbidity and mortality among transplant recipients along with respiratory syncytial virus, and parainfluenza [6, 7].

The incidence of influenza infections in HCT recipients is 1.3–2.6%, and significantly higher rates may be seen during the peaks of influenza outbreaks in the community [8]. In SOT recipients, the incidence of influenza infection varies depending on the type of organ transplanted; it is higher among lung transplant recipients than among recipients of other solid organs [9]. In a study involving 3569 SOT recipients, the incidence of influenza infection was 41.8 cases per 1000 person-years after lung transplants, 4.3 cases per 1000 person-years after kidney transplants, and 2.8 cases per 1000 person-years after liver transplants [10] and most studies reported an incidence between 2% and 4% [11–13].

Recent studies reporting on the 2009 H1N1 influenza pandemic have greatly increased our knowledge of the epidemiology of influenza infection in the transplant population [14]. A steady decrease in complication rates has been seen over the past decade, likely for several reasons, including improved supportive care, more sensitive and rapid diagnostics that enable earlier treatment, and better treatment strategies [15].

30.1.2 Clinical Presentation and Prognostic Factors

Influenza is an acute, usually self-limited, febrile illness. The clinical presentation of influenza infection in SOT and HCT recipients does not differ substantially from the typical "flu-like" illness described in the general population [11, 16, 17]. However, transplant recipients may have either atypical presentation limited to shortness of breath or weakness [7] or only constitutional symptoms, sometimes without a fever [12] (Table 30-1). These possibilities reinforce the importance of a high index of suspicion, especially during the respiratory season. In both SOT and HCT recipients, infection with influenza virus may present either with upper respiratory tract infection (URI) or lower respiratory tract infection (LRI), and appears to produce the severest symptoms in the early posttransplant period (<3 months) [8, 9, 13].

In HCT recipients, URI symptoms may consist of rhinitis and cough. Fever is more often described in patients with pneumonia (LRI). Clinical symptoms reported less frequently are muscle aches, sore throat, dyspnea, and sometimes gastrointestinal and neurological symptoms [8, 18].

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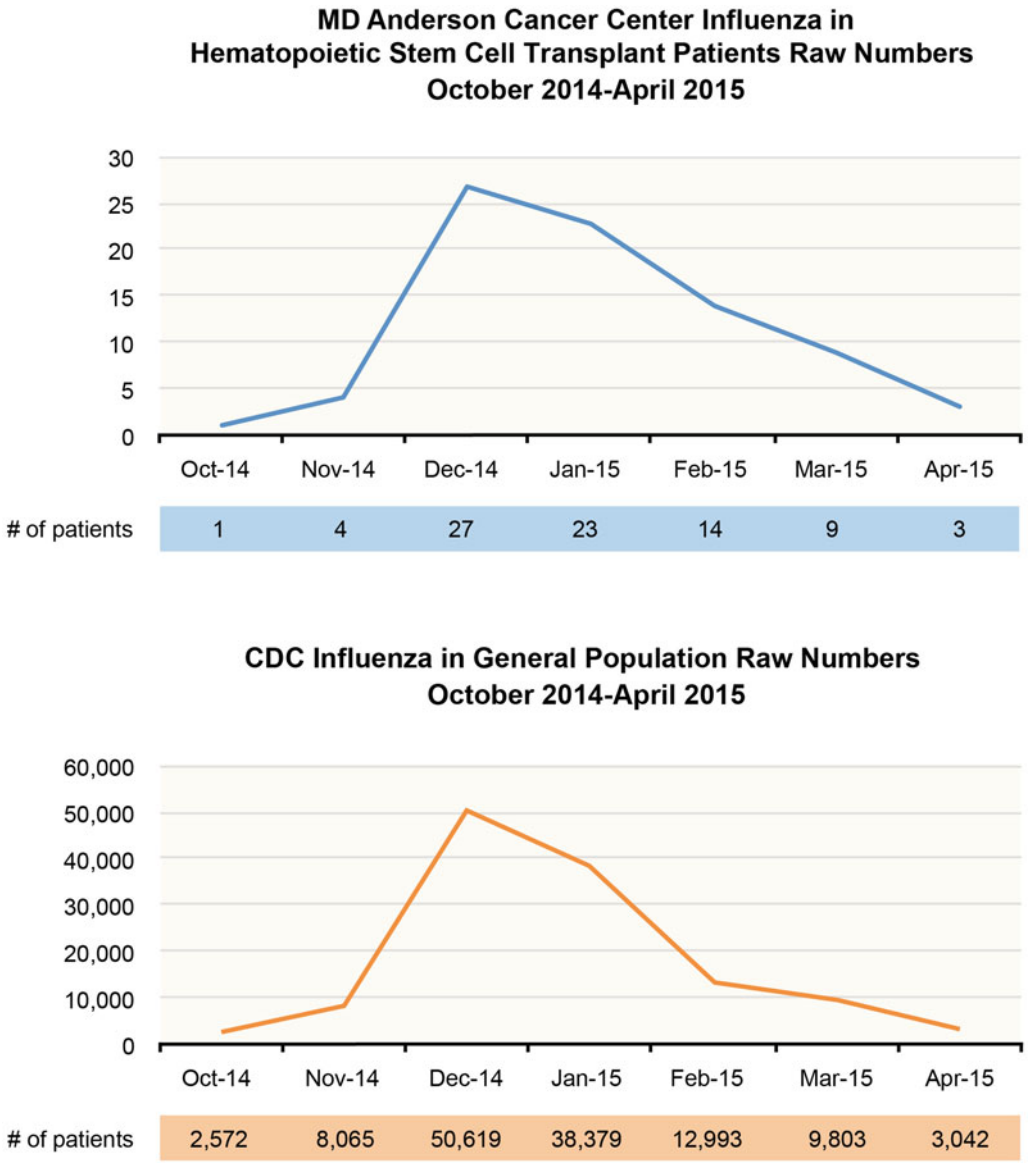

FIGURE 30-1. Centers for Disease Control Outpatient Illness Surveillance Weekly Summary for 2014–2015, showing Influenza Positive Test, National Summary. (Data obtained from http://www.cdc.gov/flu/weekly/index.htm#whomap. Accessed July 25, 2015.) Comparison with MD Anderson Cancer Center Flu surveillance. (Data provided by Dr. Roy Chemaly, Infection Control section at MD Anderson Cancer Center).

TABLE 30-1. Comparison of clinical presentations of Influenza and Parainfluenza viruses in Hematopoietic Stem Cell and Solid Organ Transplant recipients

Clinical presentation	Viral infection	
	Influenza [17–20]	Parainfluenza [21–24]
Most common symptoms	Fever, cough, rhinorrhea	Croup, conjunctivitis (better characterized in children) High fever, cough, coryza
Common symptoms	Muscle ache, sore throat, headache, dyspnea	Rhinorrhea, and/or sore throat
Rare symptoms	Gastrointestinal symptoms, neurological symptoms, fatigue	Pneumonitis in absence of upper respiratory infection Parotiditis, epiglottitis, myocarditis, and pericarditis

In cases of LRI, radiographic abnormalities may include typical diffuse ground glass infiltrates, which occur in 14–49% of patients, or air space consolidation resembling fungal or bacterial disease (Figure 30-2) [15, 18, 25].

Studies have investigated many risk factors associated with the severity of influenza infection and post-influenza complications in HCT recipients, including factors associated with the progression to LRI (Table 30-2). LRI occurs in

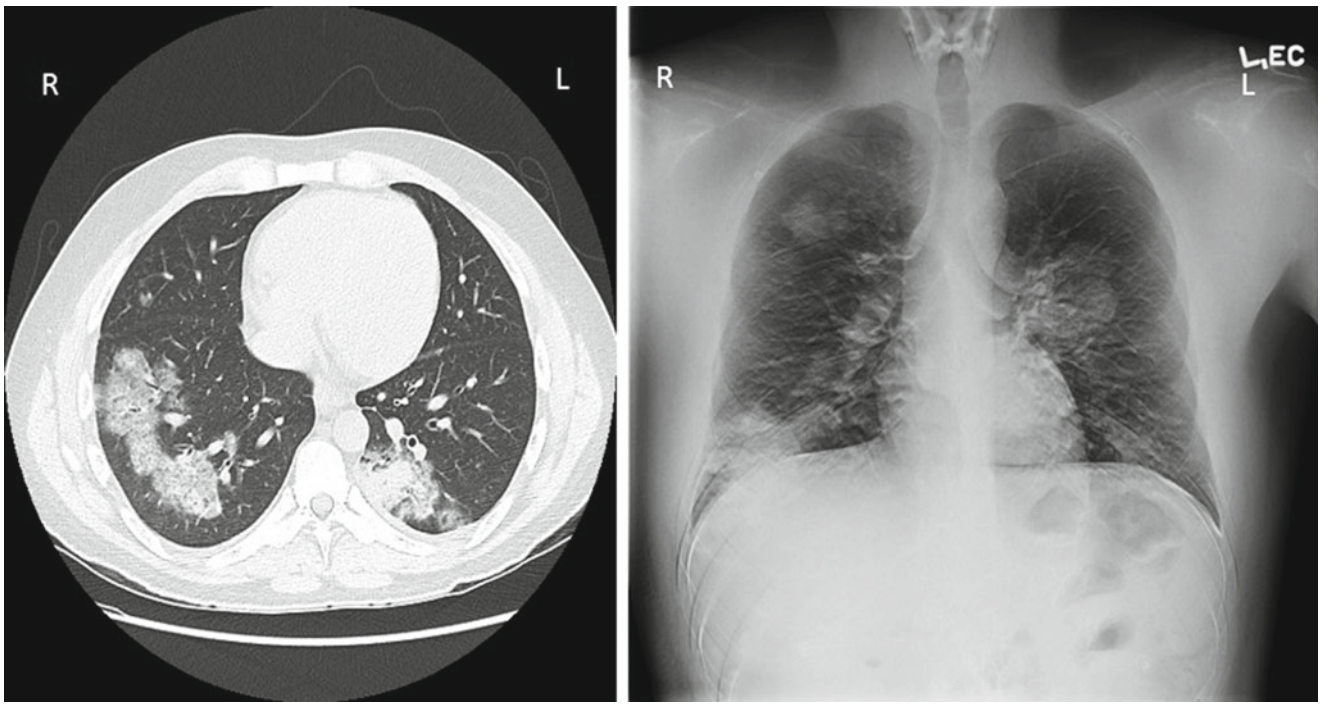

FIGURE 30-2. 29-year-old male with Hodgkin lymphoma status post-Haploidentical HCT Admitted with myalgias, cough, shortness of breath, headache. Nasal wash PCR positive for Influenza A. Chest radiograph (*right*) and computed tomography scan of the chest (*left*), showing bilateral multifocal infiltrates with consolidative pattern. Abbreviation: *HCT* stem cell transplant.

about 30–50% of HCT patients with influenza [18, 19, 36] and usually develops within a week of the onset of symptoms [8, 11]. At The University of Texas MD Anderson Cancer Center, factors associated with progression to LRI were profound lymphocytopenia, defined as an absolute lymphocyte count of <200 cells/mL (odds ratio [OR], 2.85; 95% confidence interval [CI], 1.01–8.09; $P=0.049$); age older than 65 years (OR, 2.76; 95% CI, 1.34–5.73; $P=0.004$); and neutropenia, defined as an absolute neutrophil count of <500 cells/mL (OR, 2.06; 95% CI, 1.13–3.75; $P=0.016$) [11]. Other studies have also identified lymphocytopenia as a risk factor for progression, although using different upper cutoffs (100–300 cells/mL) [13, 18, 20, 27, 29]. Finally, early antiviral therapy for URI within the first 48 h of presentation has been associated with better prognosis in several studies [8, 29, 31, 36]. Furthermore, studies of HCT recipients with 2009 A/H1N1 infections found that presentation with LRI, age >65 years, and nosocomial acquisition were risk factors for the need for mechanical ventilation [18, 29]. The use of corticosteroids at 1 mg/kg was reported by some authors to have an inverse association with need for mechanical ventilation and progression to pneumonia [29, 37], however these findings are controversial as publications from Fred Hutchinson Cancer Research Center and others, have associated steroid therapy with prolonged viral shedding and in some instances progression to LRI [8, 13, 31]. Moreover, the experience of intensive care departments during the 2009 H1N1 influenza pandemic showed that steroid therapy did not result in better outcomes and was associated with increased risk of super infections, also argues against corticosteroid use [38].

Influenza fatalities in HCT recipients have mostly been associated with respiratory complications, including need for mechanical ventilation, with mortality rates of 6–28% [8, 29, 36, 39]. Mortality can be attributed either to the direct effect of influenza viral infection with subsequent respiratory failure or to superimposed infections with concomitant pathogens, including bacteria, fungi such as *Aspergillus fumigatus*, and other viruses such as RSV [11, 36, 39].

Most of our knowledge of influenza in SOT recipients is derived from reports on the 2009 H1N1 pandemic [17]. However, from studies previous to 2009, presentation with fever in addition to respiratory symptoms strongly suggested influenza infection, as opposed to other viral infections, in SOT recipients [9]. During the 2009 pandemic, constitutional symptoms (e.g., fever, fatigue, malaise, myalgia) and URI symptoms (e.g., rhinorrhea, sore throat) were frequently described, while gastrointestinal (diarrhea), central nervous system (encephalitis), heart (myocarditis), or skeletal muscle (myositis) symptoms were comparatively rare [16, 40] (Table 30-1).

Studies have shown that rates of complications in SOT recipients during the 2009 H1N1 pandemic were generally higher than those observed during previous seasonal influenza outbreaks. Two studies conducted in SOT recipients before 2009, including mostly pediatric SOT recipients and adult renal transplant recipients; in general, they portrayed

TABLE 30-2 Factors associated with progression to Lower Respiratory tract Infection (LRI) in Hematopoietic Stem Cell and Solid Organ Transplant recipients with Influenza and Parainfluenza infections

Factors associated with progression to LRI in hematopoietic stem cell transplant recipients			
Influenza		Parainfluenza	
Study	Significant Factors	Study	Significant Factors
Ljungman et al., 2001 [13] *N*=16	Lymphocytopenia (<200 μg/L) Neutropenia (<500 μg/L)	Nichols et al., 2001 [23] *N*=253	Glucocorticoid therapy (dose-dependent) Donor cytomegalovirus serostatus positive Lymphocyte count
Nichols et al., 2004 [8] *N*=62	Lymphocytopenia (<100 μg/L) Glucocorticoid associated to prolonged viral shedding	Chakrabarti et al., 2002 [26] *N*=125	Allogeneic transplant Time from transplant to infection<6 months
Martino et al., 2005 [27] *N*=39	Alternative donor Graft-versus-host disease Lymphocytopenia (<200 μL/L)	Srinivasan et al., 2011 [28] *N*=46	Time from transplant to infection ≤100 days Glucocorticoid therapy Absolute leukocyte count <100 cells/μL <mu>at infection onset
Chemaly et al., 2006 [11] *N*=112	Age>65 years Neutropenia (absolute neutrophil count (<500 cells/mL)) Lymphocytopenia (absolute lymphocyte count (<200 cells/mL))	Chemaly et al., 2012 [21] *N*=80	Neutropenia (neutrophil count <500 μg/L <mu>) within 1 week of diagnosis APACHE II score >15 Respiratory coinfections
Khanna et al., 2010 [20] *N*=19	Lymphocytopenia (<100 μg/L)[a]	Ustun et al., 2012 [22] *N*=173	Age at presentation, 10–19 years MMRD Graft-versus-host disease Glucocorticoid therapy Coinfections Time from transplant to infection <3 months
Choi et al., 2011 [29] *N*=62	Lymphocytopenia (<100 cells/mL) Hypoxemia	Seo et al., 2014 [30] *N*=544	Time from transplant to infection <365 days Oxygen use at diagnosis Monocyte count (<100 cells/μL) Neutrophil count (<1000 cells/μL) Glucocorticoid therapy >2 mg/kg/day
Espinosa-Aguilar et al., 2011 [31] *N*=27	Glucocorticoid therapy >20 mg/day associated with development of LRI Trend associated of lymphocytopenia (<500 cells/mL) and LRI		
Factors associated with progression to LRI in solid organ transplant recipients			
Influenza		Parainfluenza	
Kumar et al., 2010 [17] *N*=237	Delayed antiviral therapy Shock at presentation Diabetes mellitus	DeFabritus et al., 1979 [32] *N*=16	Good outcomes
Smud et al., 2010 [33] *N*=77	Delayed antiviral therapy Presentation with LRI	Wendt et al., 1992 [34] *N*=19	Age <18 years
Cordero et al., 2012 [14] *N*=77	Diabetes mellitus Time from transplant to infection <90 days Septic shock Bilateral pulmonary involvement Time from onset of symptoms to initiation of antiviral therapy	Apalsch et al., 1995 [35] *N*=42	<6 months after transplant Augmentation of immunosuppression Time from transplant to infection ≤1 month

Abbreviations: *LRI* lower respiratory tract infection, *PIV* parainfluenza virus, *APACHE* Acute Physiology and Chronic Health Evaluation, *MMRD* mismatched related donor, *URI* upper respiratory tract infection, *RI* lower respiratory infection.
[a]Associated with longer viral shedding.

milder, more self-limited illnesses than those described in patients infected with the 2009 H1N1 influenza virus [9, 16]. In contrast, Kumar et al.'s report, on the 2009 pandemic which included 237 SOT recipients from different centers, described a high incidence of pneumonia (31.7%,) and higher rates of admission to intensive care units (ICUs) (15.6%) [17]. Other publications from the 2009 pandemic mirrored these findings, with rates of influenza-associated LRI in SOT recipients as high as 49%, and ICU admission rates of 10–16%, mostly related to respiratory failure [10,

14]. Factors associated with progression in SOT recipients included delayed initiation of antiviral therapy (OR, 3.03; 95% CI, 1.24–7.39; $P=0.015$) [17] and influenza infection occurring within 90 days of transplant (OR, 5.00; 95% CI, 2.10–12.20; $P=0.02$) (Table 30-2) [14, 33].

Influenza-related mortality rates in SOT recipients range from 4% to 9% [9, 10, 41]. Early therapy with oseltamivir has been associated generally with decreased rates of mortality and ICU admission and fewer complicated outcomes in SOT recipients [15, 17]. As in HCT recipients, presentation with pneumonia (OR, 21.6; 95% CI, 2.9–155.8; $P<0.001$) [15] and bacterial, viral, or fungal coinfections are relatively common (incidence, 7–29%) in SOT recipients; these coinfections have been associated with a higher risk of death [15, 17].

30.1.3 Diagnosis

The availability of rapid and sensitive methods to diagnose respiratory viral infections, and influenza in particular, not only allows early diagnosis and initiation of therapy but also limits the risk of nosocomial transmission by facilitating appropriate use of antibiotics and rapid implementation of isolation precautions when necessary [42]. Reliable and accurate diagnosis depends on the quality of the respiratory samples collected for laboratory testing. Nasopharyngeal aspirations, nasal washings, and bronchoalveolar lavages are the optimal specimens, though throat and nasal swabs have been used and can also provide an accurate diagnosis [43]. Respiratory specimens should be collected and placed in viral transport media, preferably at 4 °C, as infectivity is lost at higher temperatures. If a delay of more than 24 h before processing is anticipated, specimens should be frozen [44].

30.1.3.1 Antigen Detection Assays

Antigen detection assays include the enzyme-linked immunosorbent assay (ELISA), immunofluorescence assay, and enzymatic detection by chemical reactions. These tests have quick turnaround times—as fast as 15 min—and do not require specialized laboratory equipment or personnel; thus, they can be used outside certified laboratories in point-of-care settings. Most point-of-care tests distinguish influenza A virus from influenza B virus, but they do not consistently identify avian influenza virus subtypes. Furthermore, these tests have more variable sensitivity (47–93%) than cell culture and molecular assays, though their specificity is acceptable (around 90–95%) [45]. The results of these tests are influenced by the population, the type of specimen, and the timing of collection after presentation [46]. Their sensitivity is greatest during the peak influenza season, when false-positive results are less likely and the positive predictive value is high; however, in immunocompromised hosts, even peak-season sensitivities can be as low as 50% [47]. Thus, given the limited sensitivity of these assays, negative results do not rule out influenza virus infection, and follow-up testing with molecular testing and/or viral culture should be considered to confirm negative results, especially when clinical suspicion is high [38].

30.1.3.2 Molecular Assays

Molecular assays can identify the presence of influenza viral RNA in respiratory specimens: (a) Reverse transcription polymerase chain reaction (RT-PCR) in which purified influenza viral RNA is first reverse transcribed into cDNA, and, the cDNA is then amplified with specific primers, however this technique is time consuming and does not easily yield quantitative results. (b) Real-time quantitative RT-PCR (RT-qPCR) uses chemistry and instrumentation platforms to amplify and simultaneously quantify a targeted DNA molecule with high sensitivity and specificity as well as a rapid turnaround time. (c) Multiplex PCR is a variant of PCR that amplifies multiple DNA targets using more than one pair of primers in a single reaction tube; it is able to distinguish multiple respiratory viruses simultaneously. These assays have high sensitivity (>90%) and specificity (close to 100%) and can yield results in 3–8 h [48–50].

Some molecular assays [1] are able only to detect and discriminate between influenza A and B virus infections; while other tests can identify specific influenza A virus subtypes (A [H1N1] pdm09, seasonal A [H1N1], or seasonal A [H3N2]) [51]. Rapid molecular assays using isothermal nucleic acid amplification are a new type of molecular diagnostic test for influenza.[1] One rapid molecular assay has been approved in January 2014 in Europe, and recently in 2015 by the US Food and Drug Administration (FDA), which differentiates influenza A from B but not subtypes. It can be used in physicians' offices, emergency rooms, or health department clinics and yields results in 15 min [52, 53].

The disadvantages of these molecular techniques include their high cost, which limits their availability, and their inability to distinguish nonviable from viable viruses [54]. Detection of a weak signal may represent a commensal infection, the tail end of a previous infection, or an early evolving infection. In the case of immunocompromised patients, shedding or persistent infection may render viruses detectable by PCR for a prolonged period of time (7–45 days). Therefore, a positive test requires always to be placed into a clinical context [12, 20].

[1]The World Health Organization (WHO) information for molecular diagnosis of

Influenza virus—update can be found at http://www.who.int/influenza/gisrs_laboratory/molecular_diagnosis_influenza_virus_humans_update_201403rev201505.pdf?ua=1. Accessed 28 Aug 2015.

30.1.3.3 Cell Culture

Viral isolation by routine cell culture has been the gold standard for influenza diagnosis. However, it may take 3–10 days before the results are readily available, which may in turn have an impact on patient care [55]. The classic technique uses a series of primary cell lines (human fibroblast and rhesus monkey kidney) and continuous cell lines (A549 human lung carcinoma). The specimens are inoculated onto cell culture monolayers, and the visible cellular changes that occur in response to viral infection are monitored by light microscopy for cytopathic effect (CPE). Based on the specimen source, the time to CPE, the quality of the CPE, and the cell line(s) showing CPE, a preliminary identification of the virus can be made. The presence of a specific virus is confirmed by immunofluorescent staining using virus-specific fluorescently labeled antibodies [56]. A more rapid and sensitive viral culture is the shell vial assay, a modification of tube culture that uses a smaller tube shell vial culture. The sample is centrifuged onto a single layer of cells and viral growth is measured by antigen detection methods. The shell culture can have a turnaround time of 48 h, with sensitivity between 60% and 83% and good specificity, around 90–100% [57, 58].

Viral culture is still an important part of the diagnosis, as it allows assessment of seasonal changes, virus subtype, and potential viral susceptibilities when needed. Unlike the molecular methods and nucleic acid amplification tests, viral culture detects only viable viruses. This can be very useful for patient management because transplant recipients usually experience prolonged viral shedding, even when they are undergoing active antiviral therapy [55].

30.1.4 Management

In both HCT and SOT recipients, antiviral therapy should be initiated promptly and even empirically when influenza infection is suspected, regardless of the severity of the illness. Furthermore, antivirals should be administered even if patients present with symptoms for longer than 2 days, given the known benefit of these drugs in reducing complications [59]. In a study involving HCT recipients, virological response was seen even in patients whose treatment was started 3–6 days after initial symptoms, which is longer than the 48-h treatment window recommended for immunocompetent individuals [20]. Early administration of antiviral therapy has been associated with reduced risks of LRI (adjusted OR, 0.04; 95% CI, 00–02; $P<0001$), hypoxemia (adjusted OR, 014; 95% CI, 00–04; $P<0001$), ICU admission ($P=0.007$), hospitalization ($P=0.049$), need for mechanical ventilation ($P=0.019$), and death at 6 weeks (adjusted HR, 021; 95% CI, 00–10; $P=0049$) [21, 29]. This was also true for SOT patients in whom early initiation of antiviral therapy was associated with lower rates of progression to pneumonia (35% vs. 76%) ($P=0.013$), and delayed antiviral treatment was independently associated with increased risk of admission to ICU (odds ratio [OR] 3.03, 95% CI 1.24–7.39, $P=0.015$) [17].

Other studies in SOT and HCT recipients, as well as in patients with solid tumors have associated delay in initiation of therapy (24 h or more after onset of symptoms), with unfavorable outcomes such as progression to LRI and even death [17, 36, 60]. Most experts recommend continuing antiviral therapy until viral replication has ceased, which typically takes longer in transplant patients than the 5 days of therapy recommended for immunocompetent patients [20]. Therefore, prolonged antiviral therapy may be of benefit in transplant recipients, and experts usually recommend a 10-day course of treatment for posttransplant patients [61].

The two main groups of antivirals for influenza are the neuraminidase inhibitors [oseltamivir, zanamivir, and peramivir (licensed in some countries)], which are active against both influenza A and B [52, 62, 63], and the M2 inhibitors (amantadine and rimantadine), which only act against influenza A [64] (Table 30-3).

Oseltamivir is administered orally; its most common side effects are gastrointestinal (mainly nausea), so it is better tolerated with food. Zanamivir is administered by inhalation and is contraindicated or not well-tolerated in patients with chronic obstructive pulmonary disease and asthma (i.e., underlying airway disease). Intravenous (IV) zanamivir is undergoing evaluation in a phase 3 trial and is available for compassionate use from its manufacturer via an FDA emergent investigational new drug application in the USA [61], and compassionate use program in Europe [65]. In immunocompromised patients, increased doses of oseltamivir (150 mg twice a day, adjusted to renal function) have been used, as was recommended for patients with the H5N1 avian influenza strain. Concerns about absorption in patients with graft-versus-host disease or mucositis also motivated higher dosing. However, the benefit and impact on clinical outcomes of high-dose oseltamivir have not been conclusively demonstrated [66, 67], and we do not advocate this practice at the present time.

Peramivir is available in Japan and South Korea and has been recently approved in the USA but still under evaluation in clinical trials in Europe. Peramivir is active against influenza A and B and is indicated for the treatment of influenza infection in adults. Peramivir is the first neuraminidase inhibitor that is FDA-approved for IV use and is administered as a single IV dose [63]. It should be considered for patients who are unable to tolerate oral or enteric administration of drugs. However, further studies are needed to determine the efficacy and safety of peramivir in immunocompromised patients [62]. Lastly, Lanimavir (formerly CS-8958), which is a new-long acting inhaled neuraminidase inhibitor, has been approved in Japan in 2010 and is currently under clinical trials in the USA and Europe and promises activity against oseltamivir resistant influenza strains, but more clinical data is still required [62].

TABLE 30-3. Currently available medications for treatment of Influenza

Neuraminidase inhibitors Active against influenza A and B viruses				
Antiviral agent	Dosing	Concerns for resistance	Adverse events	Approved for prophylaxis
Oseltamivir	75 mg orally every 12 h for 10 days Requires renal adjustment Approved for children >2 weeks	Some strains of influenza A/H1N1 with H275Y mutations	Gastrointestinal, skin and neuropsychiatric events (Japanese reports) Poor absorption in severely ill patients, patients in ICU, and patients with graft-versus-host disease	Yes
Zanamivir	10 mg inhaled every 12 h (2 5-mg inhalations) Approved for children over 7 years of age	Minimal (case reports on combination with H275Y mutations and I223R and E119 neuraminidase substitutions)	Hyperactive airway; skin and gastrointestinal events	Yes
Peramivir	600 mg IV ×1 dose Requires renal adjustment Approved in Japan, South Korea and the USA for adults over 18 years of age	Potential for some strains of influenza A/H1N1 with H275Y mutation—cross-resistance with oseltamivir	Neurologic events; neutropenia; diarrhea; creatinine kinase elevation	No
Laninamivir	For aults/children age 10, a dose of 2 packages (40 mg of laninamivir octanoate) once a day. Approved in Japan. Phase 2 trials in the USA Approved in Japan Phase 2 trials in the USA	Can be used for oseltamivir-resistant influenza A strains	Gastrointestinal events	No
M2 inhibitors Active against influenza A virus only				
Antiviral agent	Dosing	Concerns for resistance	Adverse events	Approved for prophylaxis
Amantadine (generic)	100 mg orally every 12 h	Currently not recommended for treatment of influenza A (rapid emergence of resistance while on treatment)	Cardiac, neurologic, and gastrointestinal events; neutropenia	No
Rimantadine (generic)	100 mg orally every 12 h	Same as amantadine	Neurological and cardiac events	No

The M2 inhibitors (or amantadanes), amantadine and rimantadine, are not active against influenza B virus because the protein M2 is unique to influenza A viruses [46]. In addition, broad resistance to M2 inhibitors has been described; therefore, amantadanes are no longer recommended as first-line therapy for influenza in the USA unless resistance precludes the use of the neuraminidase inhibitors [68].

The use of adjunctive corticosteroid therapy remains controversial. Some studies have shown that adjuvant corticosteroids may decrease inflammation and thus prevent the development of LRI [69], but at the cost of prolonging viral shedding in immunocompromised patients or even other unfavorable outcomes [8, 70]. Thus, additional studies are needed to determine the role of corticosteroid therapy in patients with severe infection, and its use is not recommended at the present time.

30.1.4.1 Antiviral Resistance

Resistance of influenza 2009 H1N1 and H3N2 strains to the available M2 inhibitors is usually recognized early on and frequently develops over the course of treatment, particularly in immunocompromised patients [71]. The mechanisms of resistance are mostly mediated by mutations at many sites of the target pore of the M2 protein channel. Therefore, amantadine and rimantadine should not be used as first-line therapy or chemoprophylaxis for currently circulating influenza A viruses in transplant patients [68, 72].

Resistance to neuraminidase inhibitors among the influenza viruses is an emerging problem of serious epidemiological and clinical implications. A recent meta-analysis, including 19 studies, reported a pooled incidence rate for oseltamivir resistance of 2.6% [73]. However, higher rates of resistance (up to 16.3%) have been reported from Japanese children in 2004 [74]. Furthermore, during the 2007–2008 season in Europe, increased resistance to oseltamivir (up to 17%) was found to be associated with a specific mutation in the seasonal influenza A/H1N1 virus strains, H275Y, which causes a histidine to tyrosine substitution in the neuraminidase [75]. These resistant strains have been reported worldwide [76, 77] and have elicited concern because they have caused severe clinical disease in patients with cancer, with the potential for nosocomial spread [78]. Strains with H275Y mutations were also reported during the 2009 H1N1

pandemic, and some of these mutations arose after treatment with oseltamivir [78, 79]. Finally, although rare, some instances of oseltamivir-resistant H3N2 strains have been detected worldwide [64]. In immunocompromised patients, the extended duration of viral replication and shedding and the prolonged use of antiviral agents for chemoprophylaxis may explain the development of oseltamivir-resistant influenza A viruses, especially those with H275Y mutations [80, 81].

A small number of oseltamivir-resistant 2009 H1N1 viruses have also shown reduced peramivir susceptibility [82, 83], generating great concern about the use of peramivir in immunocompromised patients with oseltamivir-resistant influenza A infections. Therefore, patients infected with influenza A virus that is suspected or documented to have the H275Y mutation should not be treated with peramivir [78, 84]. The treatment of choice for oseltamivir-resistant influenza with the H275Y mutation is zanamivir, and few reports of good outcomes with the use of zanamivir in HCT recipients have been published [85, 86]. However, it is important to mention that zanamivir-resistant strains of 2009 H1N1 influenza virus have been reported in immunocompromised patients after prolonged antiviral use. These strains showed specific mutations in the neuraminidase (I223R neuraminidase substitutions), occurring in combination with or after the H275Y substitution, that have been associated with reduced susceptibility to zanamivir [87, 88]. Therefore, patients undergoing influenza treatment who do not have an appropriate clinical response within 3–5 days of the initiation of antiviral therapy and/or have a worsening course despite ongoing therapy should raise suspicion of an infection with oseltamivir and/or zanamivir-resistant virus. Several methods for genotypic and phenotypic antiviral susceptibility testing are currently available [64, 89].

It has been suggested that triple combination therapy with oseltamivir, amantadine, and ribavirin or higher doses of oseltamivir (150 mg twice a day, adjusted to renal function) could prevent the emergence of antiviral resistance and treat severe disease [90, 91], but the efficacy and safety of these regimens still need to be determined in clinical trials [67].

30.1.4.2 Antivirals in the Pipeline

New agents for the treatment of influenza are under development. DAS181 (Fludase, Ansun BioPharma, USA) is a recombinant fusion protein that includes a sialidase derived from *Actinomycosis viscosus* that cleaves sialic acid receptors on host cells. This protein has been shown to bind to cells and efficiently remove cell-surface sialic acid residues from respiratory epithelium [92]. Sialic acid is the primary receptor for viral binding and entry into the host cell; therefore, the removal of sialic acid by DAS181 potently inhibits viral infection. Furthermore, by targeting the host cells rather than the virus, DAS181 may be less likely than virus-targeted drugs to induce resistance [93]. In a phase 2 study, DAS181 significantly reduced viral load in participants infected with influenza [93]. More efficacy studies are needed to investigate clinical outcomes and the effect of the medication on viral load and shedding.

Favipiravir (T705, Toyama Chemical, Japan) is an investigational antiviral drug that functions as a nucleotide analogue and inhibitor of the viral RNA polymerase of influenza types A, B, and C, including oseltamivir-resistant strains [64]. Synergy with oseltamivir has been demonstrated in preclinical models [94, 95], and is currently undergoing Phase III clinical trials in the USA, Europe, and Latin America. Nitazoxanide, an antiparasitic agent with a novel antiviral effect [96], has been tested in healthy adults and has been shown to reduce the duration of symptoms after acute uncomplicated influenza infections [97]. In view of the ongoing drift or shift of the influenza A virus and the possibility of new pandemics or serious epidemics, future studies are needed to evaluate combination therapies and even newer agents, such as neutralizing monoclonal antibodies [98] and Toll-like receptor agonists, that elicit broad-spectrum immune response against influenza viruses [99].

30.1.5 Prevention

Person-to-person transmission of the influenza virus may occur through small aerosols, large droplets, or direct and indirect contact. The two basic tenets of influenza infection prevention, therefore, are strict attention to hand hygiene and social distancing [100]. Patients with known or suspected influenza infection should be isolated from other patients using standard and droplet precautions, which include the use of gloves, gowns, and surgical masks when close contact is anticipated and goggles or face shields when appropriate, in particular during aerosol-generating procedures [101].

In general, individuals with influenza infection may become infectious 1 day before symptoms' onset and up to 7 days after becoming sick; however, because immunocompromised patients may have prolonged shedding of the virus, they may be contagious for longer than 7 days. Nosocomial transmission of influenza virus is underestimated, but several outbreaks in transplant and non-transplant units have been reported [81]. Early and accurate diagnosis, by prompting isolation precautions, is of tremendous importance in preventing nosocomial transmission. Other precautionary measures should be established in outpatient settings, where transmission may occur in waiting areas. Screening patients for respiratory symptoms during the winter season at the front desk or at check-in and providing masks and alcohol-based gel for hand hygiene may reduce transmission of influenza virus. In addition, health care workers with flu-like symptoms or with potential respiratory viral infection should be furloughed from direct patient care until influenza and other respiratory viruses are ruled out or they become asymptomatic [66].

For immunocompromised patients, daily chemoprophylaxis with strain-specific anti-influenza agents during outbreaks was recommended in the past. However, this recommendation was called into question by a randomized, double blind, placebo-controlled trial conducted during the 2009 H1N1 pandemic. This study showed that prophylaxis with oseltamivir was effective in reducing influenza burden by 75% in high-risk transplant recipients, but at the price of an increase in oseltamivir-resistant influenza A strains [76, 102–104]. At our institution, we recommend providing empiric therapy for immunocompromised patients with upper respiratory symptoms during the influenza season until molecular testing results by PCR are available and reserving chemoprophylaxis in case of nosocomial outbreaks [61].

Vaccination should be the primary means of prevention of influenza infection. The vaccine should be offered by September or October in the Northern hemisphere and by May in the Southern hemisphere [10, 105]. Several influenza vaccines are currently licensed for use, including inactivated influenza vaccines, which can be administered intramuscularly or intradermal, and live influenza vaccines, which are administered intranasal. Inactivated influenza vaccines currently in use are either trivalent (two strains of influenza A and one strain of influenza B) or quadrivalent (two strains of influenza A and two strains of influenza B). The live attenuated vaccines are contraindicated in transplant recipients and their close contacts because there is a risk of transmission of the live-attenuated vaccine strains [106]. The immunogenicity of influenza vaccine in transplant recipients is variable, depending on the type of organ transplanted, the immunosuppressive regimen used, and the composition of the preparation of the vaccine. Vaccine response in transplant recipients has been found to be lower than that observed in the general population, with antibody response ranging from 20% to 50%, especially during the first year after transplant [107, 108]. However, further studies, including a recent meta-analysis, showed that even when vaccinated patients develop influenza, the severity of the disease is reduced compared to that observed in unvaccinated patients [109–111]. Inactivated influenza vaccine is therefore recommended for all HCT and SOT recipients as well as their household members [10, 109, 112, 113]. Vaccination is usually offered 6 months after transplantation or as early as 4 months after transplantation in case of community outbreaks [112]. Ensuring compliance with vaccination in HCT and SOT recipients and their family members has been challenging, though compliance rates have improved over the years [114, 115]. A patient survey at MD Anderson Cancer Center showed that a strong recommendation by the clinical provider was the best predictor of compliance with influenza vaccination in an immunocompromised population [116]. Likewise, vaccination of health care workers is an essential component of protecting vulnerable patients and should be encouraged and monitored, especially in centers caring for SOT and HCT patients [117].

30.1.6 Long-Term Outcomes

After the 2009 H1N1 pandemic, several studies reported incidences of acute allograft rejection after influenza infection ranging from 9% to 61%, with the highest rates in lung transplant recipients [15]. The stimulation of cellular immunity in response to viral infection may result in enhanced recognition of allogeneic tissue, leading to acute and/or chronic allograft rejection. Furthermore, bronchiolitis obliterans syndrome (BOS), defined as new onset of obstructive disease after transplant (measured by a decline in forced expiratory volume [FEV1] in 1 s), has been described after influenza infection in HCT and lung transplant recipients. However, the relationship of influenza infection with these outcomes is not yet as clear as it is for other viruses, including RSV and parainfluenza virus (PIV), and requires further study [118, 119].

30.2 Parainfluenza Virus Infection in Hematopoietic Stem Cell and Solid Organ Transplant Recipients

30.2.1 Overview and Epidemiology

PIV is a medium-sized, enveloped, single-stranded RNA virus belonging to the *Paramyxoviridae* family. PIVs are divided into two broad classes and four types based on their genetic and antigenic characteristics: respirovirus (PIV-1 and PIV-3) and rubulavirus (PIV-2 and PIV-4). In the general population, most clinical infections are caused by PIV-3 (52%), followed by PIV-1 (26%) and PIV-2 (12%) [120]. Although PIV infections often occur year-round, peak seasonal activity has been reported to occur biennially between late September and December for PIV-1, and during the spring and summer months for PIV-3 [22, 120].

The vast majority of PIV infections occur in infants and children and PIV accounts for 30–50% of the cases of croup (acute tracheobronchitis) [121]. By adulthood, more than 90% of individuals have antibodies to PIV, though they are only partially protected against subsequent infections. Even in the presence of high levels of serotype-specific antibodies, primary infection and reinfection can still occur [12]. Besides children, immunocompromised patients, including HCT and lung transplant recipients, are among the most vulnerable to PIV infections [11, 122]. As in the general population, PIV-3 is the virus type most commonly observed in transplant recipients [23].

In HCT recipients, the incidence of PIV infections has generally been reported to be in the range of 1–7% [21, 61], but a recent report of surveillance in asymptomatic HCT recipients found a much higher incidence of 18% [12]. At MD Anderson Cancer Center, a higher rate of PIV infection in allogeneic transplant recipients (5.5%) was reported when

comparing with autologous population (1.3%) [21]. Most PIV infections occurred during the first year after transplant [123], and in a study from the University of Minnesota where more than half (58%) occurring within the first 100 days [22]. It is possible that the reported high rates of nosocomial transmission, which range from 13% to 41% in different centers, have been in part responsible of early occurrence of PIV after stem cell transplant [12, 21].

In studies from the 1990s, PIV infection in HCT recipients was shown to be strongly related to young age [34, 124]. For example, in Wend et al.'s 1995 report [124], 55% of HCT patients infected with PIV were younger than 18 years of age. More recently, however, the average age of presentation has shifted upward. In Ustun et al.'s longitudinal study [22], which tracked the epidemiology of PIV in HCT recipients for over 30 years, patients 19 years old or younger made up 80% of PIV cases from 1974 to 1992, but only 12% from 2002 to 2010. Similarly, in an MD Anderson Cancer Center study, including data from 2002–2007, the mean age of presentation of PIV infection in over 200 patients with leukemia or after HCT was around 54 years [21].

Limited data are available on PIV infections in SOT recipients. The incidence of PIV infection in SOT patients varies between 1.6% and 11.9%; most of the reported infections have been in lung transplant recipients [125, 126]. The wide difference in the reported incidence rates can be explained in part by the method of detection, time of year, geographic location, and, of course, the type of organ transplanted [122]. Importantly, the majority of PIV infections in lung transplant recipients are described between the first and second years after transplant, mainly reflecting exposure in the community [16, 119, 126].

30.2.2 Diagnosis

As there are no clinical features pathognomonic for PIV infection, microbiological diagnosis is of paramount importance. Viral culture is the gold standard for diagnosis of PIV. The preferred cell lines for PIV culture are monkey kidney cell lines (e.g., LLC-MK2), but commercially available mixed cell culture systems will support growth as well. The culture usually takes 2–10 days; therefore, its clinical value is limited [24]. More rapid diagnostic methods such as antigen detection and molecular testing are commercially available and should be used for immunocompromised patients in particular.

Antigen testing for PIV can be performed with immunofluorescence indirect assays using antisera against each of the PIV serotypes, ELISA, radioimmunoassay. The sensitivity of these tests has been reported as ranging from 28% to 84% [44]. In a study by Sable et al., viral isolation and antigen detection assays using upper respiratory tract specimens showed low sensitivity for PIV diagnosis when compared with tests using specimens obtained by bronchoalveolar lavage [127]. These findings can be explained in part by the relatively low sensitivity of these tests in general and the fact that PIV in adult patients may be shed in low titers [128]. The specificity of immunofluorescence assays, however, has been reported to be approximately 95% [24].

Molecular tests, including PCR techniques, have a sensitivity of 100% and specificity of 95–98% [129]. Multiplex RT-qPCR assay kits using a dual-priming oligonucleotide system are now available; this kit is capable of detecting 12 common respiratory viruses. When interpreting molecular test results, however, it is important to keep in mind that prolonged viral shedding has been reported in immunocompromised patients, including asymptomatic HCT patients [12].

Radiological findings are not pathognomonic, though non-cavitary pulmonary nodules, especially peribronchial ground glass infiltrates and consolidation, have been reported [130].

30.2.3 Clinical Presentation and Prognostic Factors

HCT recipients with PIV infections may present with fever, cough, wheezing, and coryza [34, 85]. Sinusitis may be seen in around 40% of patients [123]. Parotiditis, epiglottitis, and even dissemination to the brain, myocardium, and pericardium have also been reported [131, 132] (Table 30-1). Interestingly, a study by Peck et al. [12] showed that up to 17% of HCT recipients tested positive for PIV when weekly respiratory samples were collected, regardless of whether they showed respiratory symptoms.

Two large studies at Fred Hutchinson Cancer Research Center and MD Anderson Cancer Center demonstrated that the most common presentation of PIV infections in HCT recipients was URI (57–87%), followed by URI and LRI (30%), and LRI without URI (7–15%) [51, 60]. In a prospective study at 37 centers in Europe, progression from URI to LRI occurred in 13–49% of patients; radiographic findings varied and included focal or diffuse interstitial and alveolar interstitial infiltrates (Figure 30-3). Most infections progressed within the first 7 days of presentation [23, 36]. Factors that were associated with progression included low neutrophil count (<500 μg/L) (OR 4.3; 95% CI, 1.9–10.0; $P<0.001$); respiratory coinfections within a month of PIV infection (OR 7.1; 95% CI, 2.7–18.8; $P<0.0001$) [21]; low lymphocyte counts (<200 μg/L) (OR 1.73; 95% CI, 1.1–2.9; $P=0.032$) [23, 30]; and the use of glucocorticoid therapy, dose dependent as follows: dose of 1–2 mg/kg/day (OR, 8.6; 95% CI, 2.6–27.8; $P=0.0003$) and dose >2 mg/kg/day (OR 19.8; 95% CI, 5.2–74.6; $P<0.0001$) [22, 30] (Table 30-2).

Respiratory failure secondary to PIV is a significant and common complication in HCT recipients; it may occur in 19–43% of patients with pneumonia [21–23]. Mortality rates range from 25% to 46%, with higher rates reported in patients who develop LRI (HR, 2.6; 95% CI, 1.3–5.4,

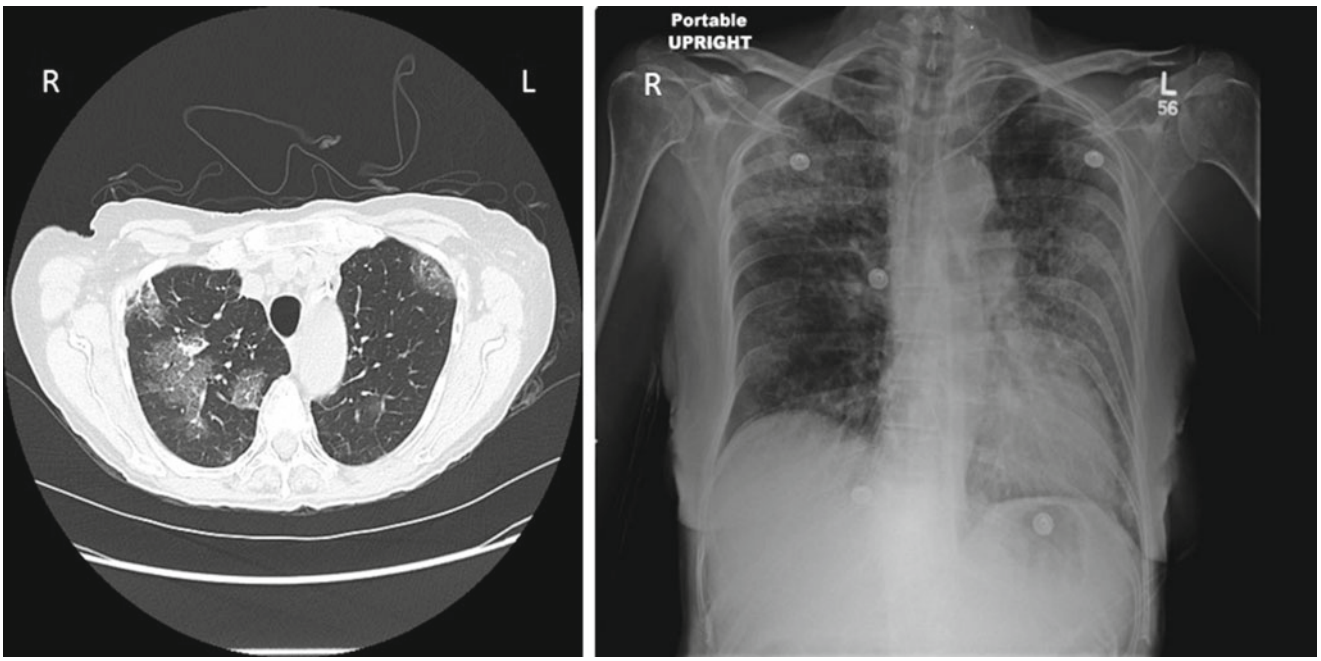

FIGURE 30-3. 68-year-old female with AML DAY+255 Matched-unrelated transplant. Admitted with nasal congestion, cough, dyspnea and fever. Nasal wash was positive for PIV 3. Chest radiograph (*right*) and computed tomography scan of the chest (*left*) showing bilateral ground glass opacities. Abbreviations: *AML* acute myelogenous leukemia, *PIV* parainfluenza virus.

$P<0.01$); who present at age 10–19 years (HR, 4.2; 95% CI, 1.8–9.9; $P<0.01$); who received transplants from a mismatched related donor (HR, 3.8; 95% CI, 1.7–8.5; $P<0.01$); and who developed PIV infection within 3 months after transplant (HR, 2.3; 95% CI, 1.2–4.3; $P<0.01$) [22]. The presence of pulmonary coinfections secondary to *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Stenotrophomonas maltophilia* (HR, 2.1; 95% CI, 1.0–4.2; $P=0.04$) and steroid use (HR, 2.0; 95% CI, 1.0–3.9; $P=0.05$) were also associated with higher mortality rates [22, 30, 133].

Reports of PIV in SOT recipients are scarce. Some retrospective studies in adults and children evaluating respiratory virus infections after SOT have reported PIV and influenza to be the most common viruses isolated, with an overall low risk for LRI; however, these infections may be associated with acute allograft rejection [32, 35]. Reported clinical symptoms of PIV infection in SOT recipients are listed in Table 30-1. Vilchez et al. [125] described the clinical presentation of PIV infections in lung transplant recipients as nonspecific, including cough, wheezing, coryza, shortness of breath, and temperature elevation above 38 °C [125]. Various studies have reported time to symptom onset after lung transplant ranging from 260 days to 2 years, mostly reflecting exposure in the community [124, 125]. From 10% to 66% of SOT patients with PIV developed LRI; risk factors for progression included previous anti-lymphocyte therapy, lymphocytopenia, corticosteroid use, and time from transplant to infection (≤1 month) (Table 30-2) [7, 35, 122]. Acute respiratory failure requiring mechanical ventilation occurred in 10–21% of SOT recipients and is associated with a higher risk of death [125, 134]. Reported mortality rates from PIV infection among lung transplant recipients vary from 8% to 20% and are strongly associated with the presence of coinfections with *S. aureus* and *P. aeruginosa* [21, 125].

30.2.4 Management

There is currently no antiviral agent licensed for the treatment of PIV. Management has included supportive care and, in some instances, the use of oral or aerosolized ribavirin with or without intravenous immunoglobulins (IVIGs). New antiviral agents and vaccines in the pipeline might change the paradigm of PIV infection management, particularly in immunocompromised patients.

Ribavirin is a nucleoside analogue that has broad activity in vitro against many RNA and DNA viruses. Aerosolized ribavirin is currently licensed for the treatment of severe RSV infections in young children. Oral and aerosolized ribavirin have also been used for the treatment of PIV; however, the available data remain controversial and not promising [61]. Our experience with PIV infections in HCT recipients at MD Anderson Cancer Center based on retrospective data, has shown that administration of aerosolized ribavirin did not reduce viral shedding, length of hospital stay, clinical response, duration of symptoms, or mortality rate [21]. Furthermore, in other studies there has not been major

difference in outcomes among those who were treated with aerosolized ribavirin and IVIGs from those who were treated with IVIGs alone [135]. Reports on the use of oral ribavirin in HCT recipients have, likewise, shown no impact on mortality rates when compared with supportive care alone [30, 136]. However, in lung transplant recipients with PIV infections, oral ribavirin seems to be associated with some benefits, including lower rates of BOS within 6 months of infection (5% in the ribavirin group versus 24% in the non-ribavirin group; $P=0.02$) [137, 138]. No randomized clinical trials have been conducted on the use of aerosolized or oral ribavirin in transplant patients to date.

Because the hemagglutinin-neuraminidase glycoprotein of PIV is important in membrane attachment, research has focused on the development of selective inhibitors of this major surface glycoprotein. DAS181, a novel sialidase fusion protein, has shown in vivo and in vitro activity against PIV by effectively cleaving sialic acid from respiratory epithelial cells and preventing PIV entry into the cells [61, 93]. DAS181 has been administered on a compassionate use basis for treatment of severe PIV infections in HCT and SOT recipients, with apparent clinical benefit and antiviral effects [93, 139, 140]. There is an ongoing double-blind, placebo-controlled phase 2 trial examining the effects of DAS181 in immunocompromised patients with PIV-related pneumonia. Two new antiviral molecule, BCX 2798 and BCX 2855 (BioCryst Pharmaceuticals, Inc., Birmingham, AL), hemagglutinin-neuraminidase inhibitors, are being evaluated in infected mouse models using a virus similar to PIV, recombinant Sendai virus. BCX 2798 and BCX 2855 were developed through structure-based drug design based on the structure of the lead compound, Neu5Ac2en (2-deoxy-2, 3-dehydro-N-acetyl neuraminic acid), bound to the active site of hemagglutinin-neuraminidase. Neu5Ac2en inhibits most NAs or sialidases and has been shown to reduce virus lung titers and to provide protection against lethal infection in mice [141].

The use of immunomodulation has also been of great interest in the treatment of PIV. Immunoglobulins have long been used in the management of PIV infections in immunocompromised patients, but very few human studies have evaluated the use of IVIGs to treat PIV infection in transplant recipients. In a small cohort of seven subjects with PIV infections after lung or heart-lung transplants, a combination approach using ribavirin, corticosteroids, and IVIG resulted in slower declines in lung function compared to historical controls, as measured by FEV1 [142]. In an animal study using two lots of commercial human-pooled IVIGs to treat PIV-infected cotton rats, Ottolini et al. demonstrated a significant decrease in PIV lung titers with the use of immunoglobulin G. However, they found no effect on inflammatory changes in the lungs [143].

Prince et al., also using a cotton rat model, studied the combination of intranasal topical immunoglobulin G and intranasal topical triamcinolone acetonide; showing a favorable effect on viral load and pulmonary pathology after treatment through 8 days [144]. However, when topical triamcinolone was used alone, virus titers increased more than tenfold [143, 144]. A human study found that higher doses of corticosteroid therapy in HCT recipients were associated with progression to LRI [23]. However, the mechanism of action of the corticosteroids on the pathophysiology of PIV still needs to be elucidated.

30.2.5 Prevention

PIV transmission can occur through contact with infectious fluids, either directly or indirectly through contaminated fomites or through inhalation of airborne particles. Although a few studies have examined whether PIV is transmitted by small droplets, the evidence remains inconclusive. PIV transmission can be effectively controlled by good hand hygiene, disinfection of surfaces, and environmental control of short-range transmission [145, 146]. Several outbreaks have been reported in HCT units, unfortunately due in part to the prolonged shedding of the virus, symptomatic surveillance and isolation precautions have been shown to be ineffective in terminating the outbreaks [147–149]. The recent expansion of knowledge about the need to maintain a high index of suspicion during community outbreaks, recognize asymptomatic shedding, and implement early isolation and contact precautions has reduced nosocomial infection rates from 74% to 27% in the last 30 years [22].

In view of the significant long-term implications of PIV infections in transplant recipients, efforts should focus not only on treatment but also on preventive strategies. Live attenuated PIV vaccines (rHPIV-1/84/del 170/942A) have been developed from human and bovine virus strains, and the results of phase 1 trials in infants, children, and adults suggest that they are safe and immunogenic [150, 151].

In conclusion, PIV is a common infection with high morbidity and potential mortality in transplant patients, as well as long-term effects on the graft and overall outcomes. Further research is needed, in terms of prevention and management including graft outcomes.

30.2.6 Long-Term Outcomes

PIV may activate immunological mechanisms in the lungs; it has been associated with a high rate of acute cellular rejection and risk for BOS in lung transplant recipients [118]. BOS is considered to be the single most important factor limiting long-term survival in lung transplant recipients, in part because its pathogenesis is poorly understood and treatment options are limited [125, 152]. Vilchez et al. [126] report on 24 lung transplant recipients with PIV infections described acute allograft rejection confirmed by trans bronchial biopsy in 82% of patients at the time of diagnosis and BOS in 32% of patients over 18 months of post-PIV-infection

surveillance. However, the interpretation of biopsy results may be difficult in the setting of infection because respiratory viral infections and acute rejection share similar pathological characteristics, including the presence of perivascular infiltrates [153].

In HCT recipients, BOS is one of the most common late-onset noninfectious pulmonary complications [154]. The diagnosis of BOS is based on spirometry measurements, bronchiectasis, and, in some cases, imaging showing evidence of air trapping [155]. At MD Anderson Cancer Center, 922 cases of respiratory viral infections in allogeneic HCT recipients were reviewed. Of those, 304 patients had pulmonary function tests available before and after the respiratory viral episode. The cumulative incidence of BOS was 23%, and BOS was more common following PIV and RSV infections (22%) than following influenza infections (13%) [156]. Similarly, Erard et al.'s [118] report from Fred Hutchinson Cancer Research Center documented significant airflow decline in 86% of the HCT recipients who developed PIV-attributable LRI (adjusted OR 17.9; 95% CI, 2.0–16; $P=0.01$) during the 100 days following transplantation.

30.3 Special Issues in Transplant of Influenza and Parainfluenza

Influenza transmission from organ donor to recipients, while rare, it has been documented in case reports [157, 158]. Lung and intestinal transplant recipients are at higher risk from complications from an infected donor; therefore, potential donors who died in the context of confirmed or suspected influenza infection should be ruled out. For other types of transplantation, organs can be procured as long as the donor received at least 48 h of directed therapy and the recipient is prophylactically treated with neuraminidase inhibitors [159]. Therefore, during the influenza season, a high index of suspicion should be maintained, and potential donors with URI or LRI should be microbiologically tested to rule out influenza or other respiratory viral infections, especially in cases of lung transplantation [159]. Additionally, the transplantation team should inform the potential recipient of the risks and consequences of accepting or not accepting the transplanted organ. To date, there are no reports of solid organ transplant associated PIV transmission, but as for influenza infection, high index of suspicion for donors is encouraged, including microbiological testing when indicated.

Patients who are scheduled to undergo HCT and who develop URI symptoms during the influenza season should be empirically treated for influenza while waiting for test results. A recent study from Fred Hutchinson Cancer Research Center demonstrated how symptomatic patients with upper respiratory viral infection detected before transplant (including influenza and parainfluenza) had an increased overall mortality compared with patients who had no virus detected (unadjusted HR, 3.5; 95% CI, 1.0–12.1; $P=0.05$) [160]. Thus, if at all possible, we recommend postponement of the transplant for 2 weeks or until the illness has completely resolved [61].

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31
Respiratory Syncytial Virus and Human Metapneumovirus Infection in Transplant Recipients

Christian Renaud and Janet Englund

31.1 Introduction

Respiratory viruses may cause serious morbidity and mortality in the immunocompromised host, and the transplant recipient appears particularly vulnerable. The impact of infection with respiratory viruses and the subsequent development of severe lower respiratory tract disease has been increasingly appreciated as respiratory viruses become more readily detectable. Respiratory syncytial virus (RSV) and human metapneumovirus (hMPV) are among the best-documented respiratory viruses causing a wide range of respiratory disease in transplant recipients, ranging from asymptomatic shedding to fatal respiratory failure. Understanding the epidemiology and clinical characteristics of RSV and hMPV permits clinicians to intervene pretransplant, provide appropriate infection control and prevention measures, potentially treat patients, and manage immunosuppressive therapy.

Severe respiratory disease in the seriously immunocompromised host in the mid-twentieth century was originally attributed to infection with opportunistic pathogens such as gram-negative bacteria, fungi, *Pneumocystis jiroveci*, and mycobacteria, as well as cytomegalovirus (CMV) and adenovirus. In the later twentieth century, episodes of acute upper respiratory infection (URI) and LRTI without an identified etiology were often considered to be "idiopathic" pneumonia or attributed to regimen-related toxicity or acute respiratory distress syndrome (ARDS). In the classic 1982 study of 215 non-bacterial, non-fungal pneumonias in 525 allogeneic hematopoietic stem cell transplant (HSCT) recipients, 44% of the episodes of pneumonia remained undiagnosed. The overall mortality rate associated with idiopathic pneumonia in these HSCT recipients was 60%, a strikingly high figure [1]. The potential morbidity of RSV infections was first recognized in immunocompromised children in the 1970s, more than a decade earlier than in immunocompromised adults [2–6]. The recognition of respiratory viruses as an important clinical problem likely reflects the increasing number of severely immunodeficient patients, more aggressive attempts to identify the cause of respiratory illness in high-risk patients, and the increasing ability of clinical virology and pathology laboratories to identify respiratory viruses in clinical specimens.

Although uncommon or atypical pathogens may be responsible for respiratory disease in the transplant recipient, the same viruses that cause typically mild but acute respiratory illness in the general population are responsible for hospitalizations in persons of all ages with underlying medical conditions [7]. These same viruses are also a common cause of respiratory disease in transplant recipients [8–15]. With the widespread availability of sensitive and reliable molecular diagnostic methods, RSV and hMPV have been detected worldwide in transplant recipients and shown to be common causes of respiratory disease in the immunocompromised host [16, 17]. In both the general population as well as in transplant recipients, RSV and hMPV may produce a wide constellation of clinical syndromes ranging from the common cold to bronchiolitis to severe pneumonia, but in contrast to the general population, RSV and hMPV may significantly impact the morbidity and mortality of the transplant recipient.

31.2 Virology

RSV was first identified in 1956 and became appreciated as a major cause of epidemic bronchiolitis and pneumonia in young children in the 1960s [18, 19]. HMPV was first identified in 2001 by molecular techniques in symptomatic children by van den Hoogen et al. as a paramyxovirus causing bronchiolitis and URI in children [20]. Both viruses are classified within the Pneumovirinae subfamily of the Paramyxoviridae family of non-segmented, negative-strand, enveloped RNA viruses [21]. HMPV belongs to the Metapneumovirus genus whereas RSV is a member of the Pneumovirus genus. Both viruses are highly pleomorphic and their sizes vary from 150 to 600 nm. The RSV and hMPV genomes are approximately 13–15 kb in length and closely resemble each other, excluding a few differences in the order of the genes and the absence of the non-structural

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genes (NS1 and NS2) from hMPV genome. The remaining eight genes code for nine proteins present in both viruses: the nucleoprotein (N protein), the phosphoprotein (P protein), the matrix protein (M protein), the fusion glycoprotein (F protein), the putative transcription factor (M2-1 protein), the RNA synthesis regulatory factor (the M2-2 protein), the small hydrophobic glycoprotein (SH protein), the attachment glycoprotein (G protein) and the viral polymerase (L protein). The RNA core of the virion is associated with P, N, L, M2-1, M2-2 proteins, surrounded by M protein and covered by a lipid envelope. F is the most highly conserved of the envelope glycoproteins within each virus and between RSV and hMPV. The fusion glycoprotein is essential in promoting attachment and fusion of the virus with the cell membrane during viral entry. The fusion protein is the target of many vaccines under development as well as that of monoclonal antibodies such as palivizumab, which is used to prevent RSV disease in preterm infants. By contrast, the G gene is the most variable. Whole genome analysis of both RSV and hMPV has shown the existence of two genotypes, A and B. In hMPV, those two major genetic groups are further divided into subgroups A1, A2, B1, B2 based upon the sequence variability of the G and F genes. Subgroup A2 is again divided into A2a and A2b.

RSV and hMPV infections produce both humoral and cellular immune responses. Humoral immunity protects against reinfection while cellular immunity controls established infection and terminates viral shedding. Protective immunity in immunocompetent hosts is thought to be relatively short-lived. Both viruses interfere with the host's innate immune system resulting into incomplete clearance and partial immunity.

31.3 Diagnosis

Prompt and accurate identification of respiratory viral pathogen is critically important in the transplant recipient because it enables specific infection control precautions to be instituted, the initiation of specific antiviral therapy, impacts the use of immunosuppressive therapy, and potentially affects whether transplantation should proceed [22–24]. Furthermore, identification of a respiratory viral pathogen can assist in avoiding unnecessary therapy, procedures, and surgical procedures (such as open lung biopsy), as well as assist in the identification of a potential cluster or epidemic of infections within the medical unit, hospital, or community. In hospitalized adults (both immunocompromised and immunocompetent), rapid viral diagnosis has been shown to reduce mortality and decrease the length of hospital stay and total cost [25, 26].

Laboratory diagnosis of respiratory viruses including RSV and hMPV has evolved considerably; adequate specimen collection is still essential for the successful identification of viruses in clinical samples. Newer types of nasopharyngeal swabs have shown improved viral diagnostic sensitivity compared to previous swabs, with similar sensitivity to nasal washes when using sensitive molecular methods in patients [27, 28]. For example, nylon flocked swabs and foam swabs increase cell capture within the swab and then release into the transport media, increasing viral recovery [29, 30]. Nasal wash or aspiration methods are superior for isolation of viruses by culture and increase the sensitivity of culture, antigenic assays and quantitative molecular assays [31]. Nasal washes are well tolerated in cooperative adults and offer the advantage of visualizing the quality of the specimen. Bronchoalveolar lavage remains the specimen of choice to diagnose lower respiratory tract infections because of the ability to simultaneously test for potential co-pathogens such as fungi, *Pneumocystis jiroveci*, and bacteria, as well as to document viral infection in the lower airways. Discordance in viral detection between upper and lower respiratory tract samples have been described with both viruses; negative upper tract and positive lower tract specimens in immunocompromised patients are possible but more discordance has been noted for hMPV compared with RSV.

Molecular diagnosis of RSV and hMPV is faster and more sensitive than viral culture or antigen detection, and most laboratories currently use commercial or in-house molecular assays to detect RSV and hMPV (Table 31-1). Many genes have been targeted to detect RSV, including the N, F and L genes, with similar genes also targeted to detect hMPV. Many rapid assays have been approved including some highly multiplexed respiratory panels allowing detection of RSV and hMPV as well as many other respiratory viruses and bacteria. Several different primer sets may be utilized simultaneously in the reaction mix, and the virus identified by the size of the amplicon or following hybridization with a virus-specific probe. Some commercial assays are very rapid and require minimal technical expertise, with only 1–2 h of turn around time [32, 33]. Some laboratories have developed quantitative assays using hydrolysis probe technology with standard curves to help understand the significance of positive results and to follow viral loads under therapeutic management [34–36]. No quantitative commercial assays are yet available. Molecular assays have also been used to detect viral RNA from blood/serum as a prognostic marker [37].

Unlike molecular methods, isolation of virus by culture confirms the presence of a complete infectious unit capable of further multiplication. Positive culture results may be obtained with as little as a single infectious virion, below the threshold of detection for most other detection methods, including some nucleic acid amplification test (NAAT) methods. Another advantage of viral culture is that multiple viruses may be identified from a single sample and viruses can grow independently of point mutations that could potentially create false negative results by NAAT. The major limitations of viral isolation include the time, expense, and expertise required for virus isolation.

TABLE 31-1. Diagnostic tests for common respiratory viruses

Virus	RSV	HMPV	Test advantages
Specimen	Nasopharyngeal aspirate, nasal wash, nasal swab, bronchoalveolar lavage		
Real time RT-PCR assays[a]	Widely available	Widely available	Sensitive, specific, and ability to be rapid (within 1 h); typing, determination of viral load, and sequencing possible
Enzyme-based Immunoassay (EIA[b])	Widely available	Not available in the USA but available in Canada and Europe	Rapid but less sensitive (particularly for low viral loads); relatively inexpensive
Fluorescent antigen detection	Available	Available	Less expensive, rapid; assess quality of specimen; not as sensitive as RT-PCR
Culture (clinical lab)	Central labs only	Limited labs only	Becoming less available; results take time but enables typing and analysis of viral strains

[a]Many viruses can be detected simultaneously by real-time PCR methods [32].
[b]EIA kits are available for RSV only in the USA but for hMPV outside the USA.

A variety of cell lines can be used to grow RSV (Hep-2, A549, RhMK) or hMPV (LLC-MK2, Vero), and detection by cell culture can also be accomplished using several types of cells together, such as the R-Mix cells (mixture of mink lung cells and A549 cells) [38] (Diagnostic Hybrids, Athens, OH). Centrifugation combined with viral antigen detection methods permits more rapid diagnosis [39]. RSV- and hMPV-specific monoclonal antibodies have been used for immunofluorescence (IFA) techniques either directly on respiratory specimens or in cell culture [40, 41]. The sensitivity of IFA is lower than that of NAAT for detection of RSV and hMPV. However, results can be reasonably fast, the method is relatively inexpensive, and importantly, this method also confirms that an appropriate specimen has been properly obtained by looking at ciliated epithelial cells. Sensitivity of IFA when performed by an experienced laboratory is as high as 70–90% of the samples positive by PCR—at least in children [34]. IFA can detect viruses that would be missed by NAAT because of point mutations. IFA positivity also has good clinical correlation while low grade NAAT positivity can be detected for longer periods of time with unclear significance and transmissibility.

Enzyme immunoassays (EIAs) and rapid antigenic diagnostic tests (RADTs) are commercially available for RSV and to a lesser extent for hMPV. These assays lack sensitivity and/or specificity and are not recommended in transplant populations. More than one diagnostic method should be used, since no method is perfect. Molecular assays have the greatest sensitivity—although perhaps at times can perhaps be too sensitive. Paradoxically, rare point mutations causing mismatches have been described causing false negative results of NAAT in transplant units. Viral cell culture requires time, is becoming less available in laboratories, and is more expensive and less sensitive, although it can catch those strains with mismatches and provide information on viral replicative nature while receiving treatment.

31.3.1 Strain Identification and Characterization

Further characterization of RSV and hMPV strains obtained from culture or directly from the clinical specimen is frequently desirable. Antigenic differences among virus strains isolated from different geographic locations or at different times may also be examined. Pools of monoclonal antibodies and "RNA fingerprinting" have been used in the analysis of RSV strains in nosocomial outbreaks [42, 43] but direct sequencing of the F and G glycoproteins is more commonly utilized [44, 45]. Next-generation sequencing is a very promising tool to characterize RSV or hMPV strains during severe infection. This could provide information on phylogenicity to identify outbreaks and also detect mutations that could be associated with antiviral or monoclonal resistance as well as increased virulence. Some human gene alleles in the human genome may increase RSV severity in infants, but little is known yet on these genomic variations in transplant recipients. Next-generation sequencing has the potential to provide information on the virus and the human genomes simultaneously.

31.4 Epidemiology

31.4.1 RSV Epidemiology

31.4.1.1 Hematopoetic Stem Cell Transplantation

RSV is well known to cause annual winter outbreaks in the community (Figure 31-1). Surveillance studies of respiratory viruses from transplant centers have established the high frequency and the significant clinical impact of respiratory viral infections in HSCT recipients overall [8–15, 46, 47] as well as the relative importance of RSV in terms of morbidity and mortality (Table 31-2). A 1988 retrospective review conducted at the Children's Hospital of Philadelphia revealed a

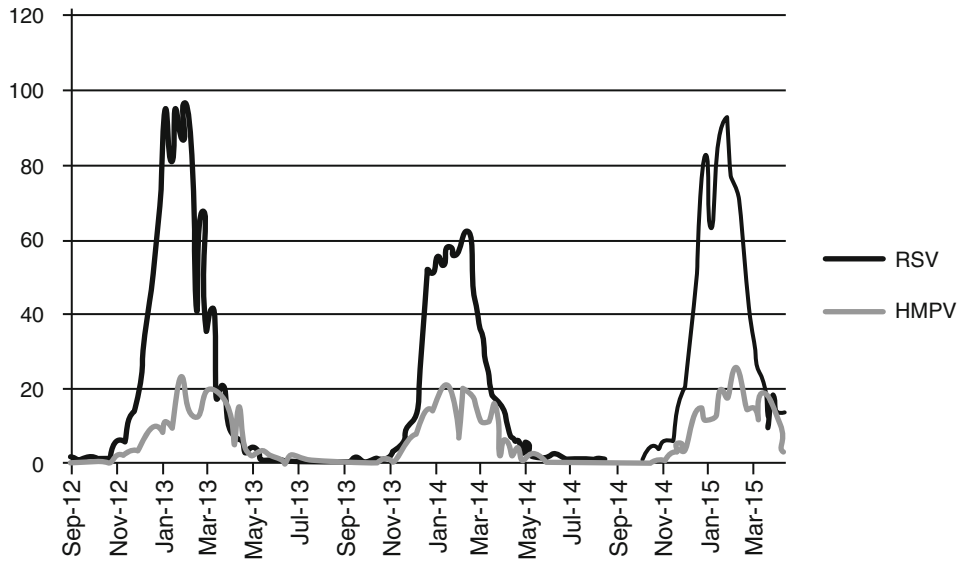

FIGURE 31-1. Seasonality of RSV and hMPV by number of cases/week in outpatient and hospitalized patients, Seattle, 2012–2015.

TABLE 31-2. Lower respiratory tract disease associated with RSV and hMPV in transplant recipients

Virus	Incidence %	Progression to LRTI (%)	Deaths associated with LRTI (%)	Reference
HSCT:				
RSV	1–12	18–55	7–43	[48, 49]
hMPV	3–7	21–40	33–40	[45, 48–50]
SOT:				
RSV-				[51–53]
Lung TX -adults	2–16%	?	10–20%	
Liver Tx (peds)	3–46%	?	12–20%	
	46%	?	?	
hMPV-				[54]
Lung TX	4%	?	33%	

respiratory viral infection in 11 (12%) of 96 pediatric HSCT patients, with only one infection due to RSV [47]. One of the earliest studies demonstrated fatal RSV pneumonia in four of 11 immunocompromised adult HSCT and solid organ transplant recipients with RSV infection [5].

Most studies conducted in the twentieth century utilized classical virological methods that were relatively insensitive for the detection of RSV and/or did not detect recently described respiratory viruses such as hMPV. Thus, these studies likely underestimated the true frequency of RSV overall. Nonetheless, early studies from large transplant centers reported serious sequelae in small numbers of patients who developed pneumonia where RSV was detected [5, 9, 13]. At the Fred Hutchinson Cancer Research Center (Hutch), a prospective surveillance study conducted in 1987 documented respiratory viral infections in 15 (19%) of 78 immunocompromised patients who were followed until hospital discharge [13]; five (33%) patients developed pneumonia and two (13%) patients died (one with RSV, the other with adenovirus). A subsequent prospective surveillance study described 127 viral infections and revealed an overall frequency of viral infections of approximately 4% [11]; 49% of RSV isolates were from BAL. This study demonstrated the relatively high numbers of patients with RSV lower respiratory tract disease, which was higher than rates of LRTI caused by PIV (22%) influenza (10%), or rhinovirus (3%).

Results of viral surveillance in transplant units vary depending on the type of surveillance protocols utilized, time of year surveillance was conducted, type of clinical samples evaluated, and laboratory tests utilized. At the Huddinge University Hospital, Stockholm, a prospective surveillance study conducted among HSCT recipients between 1989 and 1996 detected 39 (7.1%) respiratory viral infections in 545 patients, including RSV in 21% [14]. At M.D. Anderson Cancer Center (MDACC), prospective surveillance conducted using culture techniques among hospitalized adult HSCT recipients during two 6-month winter periods detected 67 (31%) respiratory viral infections in 217 hospitalized patients with acute respiratory symptom. Nearly half of these were due to RSV (33 patients, 49%). The impact of these illnesses was considerable: 20 (61%) patients had RSV infection progressing to pneumonia, and

12 patients with RSV pneumonia died. During the winter period when community viruses were frequent and nosocomial transmission was high, the frequency and mortality of respiratory viral-associated pneumonias was more than four times as high as CMV-associated pneumonia.

Recent prospective studies conducted in transplant centers worldwide continue to demonstrate the importance of RSV and report incidence of infections and risk factors for fatal disease (Table 31-2). A 2005 study from Barcelona, Spain, described the 2-year incidence of symptomatic respiratory viral infections in over 400 patients followed for up to several years posttransplant. Altogether, 29% of allogeneic HSCT recipients and 14% of autologous HSCT recipients had respiratory viruses detected, with 19 patients (4.6%) receiving either autologous or allogeneic transplants having symptomatic RSV disease [55]. Risk factors associated with having a respiratory virus identified included close household contacts with children under the age of 12 years and chronic graft versus host disease. Lymphopenia was identified as a major risk for URI progression. Similar rates have been demonstrated in other centers in the USA [15], South America [56], and Europe [57].

31.4.1.2 Solid Organ Transplantation

Less data is available documenting the impact of RSV in SOT recipients (Table 31-2), RSV has been shown to cause lower respiratory tract disease and has been associated with other complications, such as organ rejection and bronchiolitis obliterans. Lung transplant recipients have the highest rates of RSV lower respiratory tract disease in diverse types of organ transplant. RSV infections in lung transplant recipients have also been associated with organ rejection and progressive bronchiolitis obliterans, both of which have been observed to be seasonally related [58, 59]. Adult renal transplant recipients have also been reported to develop RSV-associated lower respiratory tract disease. The mortality has been low, and most patients have recovered without specific antiviral therapy [5, 60]. RSV infections in pediatric liver transplant recipients have been associated with significant morbidity and some but relatively low mortality [51]. Among 483 pediatric liver transplant recipients cared for at the University of Pittsburgh between 1985 and 1991, 17 (3.4%) children developed RSV infections, three-quarters of which were nosocomially acquired. The majority of the children had lower respiratory tract involvement, and two (12%) children died. Specific antiviral therapy was not administered. The risk factors for more severe disease included onset of infection early after transplant, preexisting lung pathology, augmented immunosuppression prompted by rejection, and younger age. Infections occurring late after transplantation in the absence of rejection were usually not severe.

An intensive prospective approach to determining the incidence and risk factors for respiratory viruses was carried out at the Hutch among 122 HSCT recipients, who were prospectively enrolled between 2000–2004 and tested weekly through 100 days posttransplant using both culture and RT-PCR detection methods [50]. The cumulative incidence estimates of hMPV and RSV at day 100 were similar, at 6.2% and 5.8%, respectively. Multivariable analysis demonstrated that only recipient CMV seropositivity was associated with increased risk for acquisition of a respiratory virus (hazard ratio = 4.1, CI 1.7–10.1, $P = 0.002$).

The frequency of RSV infections and associated morbidity and mortality differs substantially, potentially accounting for the variability reported by different institutions. These differences reflect the intensity of viral surveillances, the time of surveillance, the viruses prevalent in the community, the degree of immunosuppression of the patients, infection control policies, the inclusion of potential as well as actual transplant recipients, surveillance in outpatients as well as inpatients, the types of laboratory assays utilized, and the case definition utilized (i.e., both clinical and laboratory definitions).

31.4.2 HMPV Epidemiology

31.4.2.1 Hematopoietic Stem Cell Transplantation

HMPV has been detected worldwide with a seasonal distribution. Community outbreaks occur yearly mainly in winter and spring (January to May in the northern hemisphere; June to July in the southern hemisphere) (Figure 31-1). Often hMPV outbreaks will be concomitant with or subsequent to RSV outbreaks. HMPV most commonly affects young children less than 2 years old and is second only to RSV as a cause of bronchiolitis. Seroprevalence studies have shown a high percentage of children have contracted the virus by age 5–10 years. However, reinfection can occur later secondary to insufficient immunity or infection with different genotypes. Predominant hMPV strains can vary from location to location and from year to year. Vicente et al. have reported higher virulence by genotype A [61], while Papenburg et al. reported higher virulence by genotype B [62]. However, the interaction or impact of hMPV with other viruses or bacteria remains unclear, particularly in immunocompromised patients.

The importance of hMPV in transplant recipients has not been as well studied as RSV (Table 31-2). It was first reported shortly after the detection of hMPV by Boivin et al. [17]. An early prospective longitudinal study from Spain documented hMPV in both autologous and allogeneic HSCT recipients, with the incidence and clinical impact of hMPV and RSV disease documented to be quite similar [55]. One early prospective study documented hMPV infections in 22 adults with hematologic malignancies that progressed from upper respiratory infection to pneumonia, with a case- fatality rate close to 14% [63]. Lower respiratory tract disease and pneumonia due to hMPV infection in HSCT recipients has been reported to have an overall incidence of 1–4% [48, 55, 64, 65]. A single case series described hMPV-positive nasal

aspirate samples in 86% of 21 adults following HSCT, many of whom were asymptomatic [66]. This study demonstrated very high rates of genetically similar viruses and differs from most other studies due to high rates of genetically identical viruses. These authors suggested that these hMPV infections may have originated in the hospital nosocomially; nonetheless, this study is an outlier compared to other reports of hMPV in transplant centers.

Pneumonia rates following hMPV infection have been reported at 20–28% with mortality rates of 0–4% [67–69]. Among 163 HSCT recipients who underwent BAL for investigation of lower respiratory tract disease with pulmonary infiltrates by radiographic imaging, hMPV was detected in BAL samples from 5 of 163 (3%) patients; four of these five died with acute respiratory failure highlighting the potential severity of hMPV pneumonia [64]. A retrospective cohort study at the Hutch described a high mortality rate of 43% among patients with hMPV pneumonia, a rate similar to RSV pneumonia mortality [49]. Studies from other transplant units continue to document the potential of hMPV to cause severe lower respiratory tract disease, with clinical presentations and outcomes generally similar to RSV [49, 70].

31.4.2.2 Solid Organ Transplantation

The significance of hMPV infection in SOT recipients remains less well defined, with the exception of hMPV disease in lung transplant recipients [70]. Case reports of severe disease have been described following liver and renal transplantation [71, 72]. Rates of hMPV infection in lung transplant recipients have been reported to be similar to those seen in studies of HSCT recipients, varying from 4–6% [57, 73]. In most of these patients, hMPV appears to frequently be the sole pathogen detected. Detection of hMPV in lung transplant recipients may not necessarily signify disease, as was noted in a study of 93 lung transplant recipients undergoing BAL mainly for surveillance purposes; four cases of hMPV was detected in asymptomatic patients [74]. HMPV infection has been found in 4–6% of lung transplant recipients, but prevalence may be higher during nosocomial outbreaks [39, 75]. One study in the setting of a community outbreak identified hMPV in BAL samples from 9 of 26 (35%) patients; their clinical presentation varied from asymptomatic infection to severe disease [39].

Acute allograft rejection was more frequent in the hMPV-infected group than in the non-hMPV-infected group (33% vs. 6%, respectively; $P=0.0257$); and overall mortality was also higher (33% vs. 0%, respectively; $P<0.0025$) [39]. Another prospective study found hMPV infection as frequent as RSV after lung transplantation, and to cause as much pneumonia and acute allograft dysfunction (63% vs. 72%, respectively), but only RSV was associated with chronic allograft dysfunction at 6 months [76]. In another study, 25% of hMPV infections in lung transplant recipients were associated with acute allograft dysfunction compared with 88% for RSV [49]. A meta-analysis of hMPV respiratory infections and allograft rejection, among lung transplantation recipients indicated that detection of hMPV from airway secretions may be a significant posttransplantation occurrence. A total of 2883 samples from 1007 lung transplant recipients, were analyzed for virus detection; 337 samples had viruses identified and 57 (17%) were positive for hMPV. Twenty of these 57 (35%) cases of hMPV had acute rejection within 3 months of viral detection. There were five (9.4%) cases of chronic rejection in association with hMPV. All studies included in the meta-analysis, with the exception of one, identified rejection within 3 months. Another study has also described cases of chronic rejection within 6 months [77].

31.5 Clinical Manifestations

31.5.1 RSV in Transplant Recipients

Disease manifestations of RSV are dependent on many factors including the immunity and immune competence of the host, the time of infection related to transplant, the type of transplant, the age and underlying health of the patient, and the degree and duration of immunodeficiency. RSV infections in HSCT recipients typically follow the same clinical sequence as RSV infections in previously healthy children: signs and symptoms of a URI such as rhinorrhea, sinus congestion, sore throat, or otitis media frequently precede signs of lower respiratory tract disease including cough, wheezing, hypoxia, and pneumonia [5, 6, 9] (Tables 31-3 and 31-4). The presence of wheezing with respiratory symptoms during the respiratory virus season may provide the clue that RSV may be present. Progression of URI to LRI has been associated with patients who are early (<1 month) posttransplant,

TABLE 31-3. Clinical symptoms associated with RSV and hMPV infections in transplant recipients

Upper respiratory tract symptoms:
Rhinorrhea, congestion
Sneezing
Sore throat
Sinus congestion, sinusitis
Otitis media
Lower respiratory tract symptoms:
Cough
Wheeze
Shortness of breath, chest tightness
Systemic symptoms:
Fever
Headache
Myalgia
Hypotension

Table 31-4. Signs, symptoms and viral shedding associated with RSV and hMPV infection in HSCT recipients in a prospective single center institution[a]

Virus	Number of separate clinical episodes	*N* (%) No respiratory symptoms	*N* (%) No systemic symptoms	Viral shedding (median days)	Viral shedding (range, days)
RSV	34	1 (3)	7 (21)	11	2–76
MPV	21	0	4 (19)	24	5–100

[a]Boeckh M, Campbell A, Xie H, Kuypers J, Leisenring WM, Chien J, Jerome KR, and Englund JA. Progression, Shedding Patterns, and Clinical Disease Associated with Respiratory Virus Infections after Allogeneic Hematopoietic Cell Transplantation. Presented at American Society of Hematology Annual Meeting, New Orleans, LA; December 7–10, 2013 (Abstract 3278).

those with lymphopenia, or those who are more severely immunosuppressed. In HSCT patients who have recently engrafted, the frequency of progression to LRI tract disease ranges from 25% to 40% [78, 79] (Table 31-2) Pneumonia following RSV infection may be primarily viral, bacterial, fungal, or mixed in origin. Once RSV disease has progressed to respiratory failure, however, the mortality remains high despite the use of antiviral therapy, immunotherapy, or decreased immunosuppressive therapy. Fatality rates of RSV pneumonia range from 20% in more recent case series to over 80% in earlier studies.

Risk factors for the progression of RSV upper respiratory disease to lower tract disease or pneumonia and factors relating to fatal disease have been evaluated. The most common risk factor described in multiple centers using different methods of case ascertainment and viral detection remains lymphopenia. In a prospective multicenter study carried out by the European Group for Blood and Marrow Transplantation, lymphopenia but not neutropenia significantly increased the risk for lower respiratory tract disease [80]. Older age and donor status are also significant risk factors in some studies, whereas CMV serostatus, acute graft versus host disease, time relative to engraftment, and preemptive aerosolized ribavirin at a low dose of 2 h daily were not significant [81]. Investigators from MDACC have demonstrated that season of year, relapse of malignancy, presence of graft versus host disease, increasing age, and lack of engraftment are inpatient risk factors for the development of RSV pneumonia [9, 12, 22, 82]. Recently, large retrospective studies have identified graft source including cord or marrow (adjusted hazard ratio (HR), 4.1, 95% CI 1.8–9.0) and oxygen requirement (adjusted HR 3.3, 98% CI, 15–6.7) to be independently associated with death due to respiratory failure in HSCT recipients [83]. Smoking history, conditioning with high-dose total body irradiation, and an absolute lymphocyte count $< 100/mm^3$ at the time of URI onset are also significantly associated with disease progression [84].

Since initiation of therapy hinges on prompt diagnosis, the possibility of false negative laboratory tests must be considered in individual patients and the diagnosis should be aggressively pursued by other means, such as BAL. Regardless of the therapeutic intervention, high rates of mortality due to RSV pneumonia are documented in seriously immunocompromised patients when therapy has been initiated after the development of respiratory failure (Figure 31-2a). Survival rates remain low for severely immunosuppressed patients who are intubated due to progressive RSV pneumonia unrelated to super-imposed pulmonary hemorrhage, pulmonary edema, or bacterial superinfection [5, 50, 55, 81, 83].

31.5.2 HMPV in Transplant Recipients

HMPV may cause upper or lower respiratory tract infections in HSCT recipients. Asymptomatic shedding from upper respiratory tract has been reported, indicating that not all hMPV infections result in severe lower tract disease [50, 66, 85]. HSCT recipients with hMPV disease in the immediately posttransplant period typically present with respiratory symptoms including nasal congestion, sore throat, cough, or fever. Once lower respiratory tract disease develops, rapidly progressive pulmonary infiltrates frequently accompanied by hypotension, septic shock, or both may be present [64] (Figure 31-2b and c). In a prospective viral surveillance in HSCT recipients where samples for respiratory viruses were obtained weekly, regardless of the presence of respiratory symptoms, a cumulative incidence estimate of hMPV of 6.2% (95% CI, 1.3–11.2) over 1 year was determined; all cases of hMPV had clinical respiratory symptoms identified ranging from mild to more severe disease with single or multiple symptoms [50] (Table 31-4). Among 15 HSCT recipients with hMPV detected by RT-PCR in BAL, 10 (67%) had positive hMPV RT-PCR in nasal wash sampled within 11 days prior to or following the BAL [49]. Viral RNA was detected in the serum of one of these severely immunocompromised HSCT recipients at two time points, 4 days apart, with a viral load of ~8 Log10 copies/ml in each sample. This patient died of severe respiratory disease. In assessing risk factors associated with overall mortality at day 100 posttransplant, the use of bone marrow as the stem cell source, steroid treatment and oxygen use have been associated with overall mortality [49].

Radiographic findings associated with hMPV infection in the HSCT recipient may consist of centrilobular nodules, ground glass opacities, tree-in-bud to diffuse bilateral alveolar infiltrates [86, 87] (Figure 31-2b–f). Centrilobular nodules have been associated with less mechanical ventilation while ground glass opacities tended to be associated with

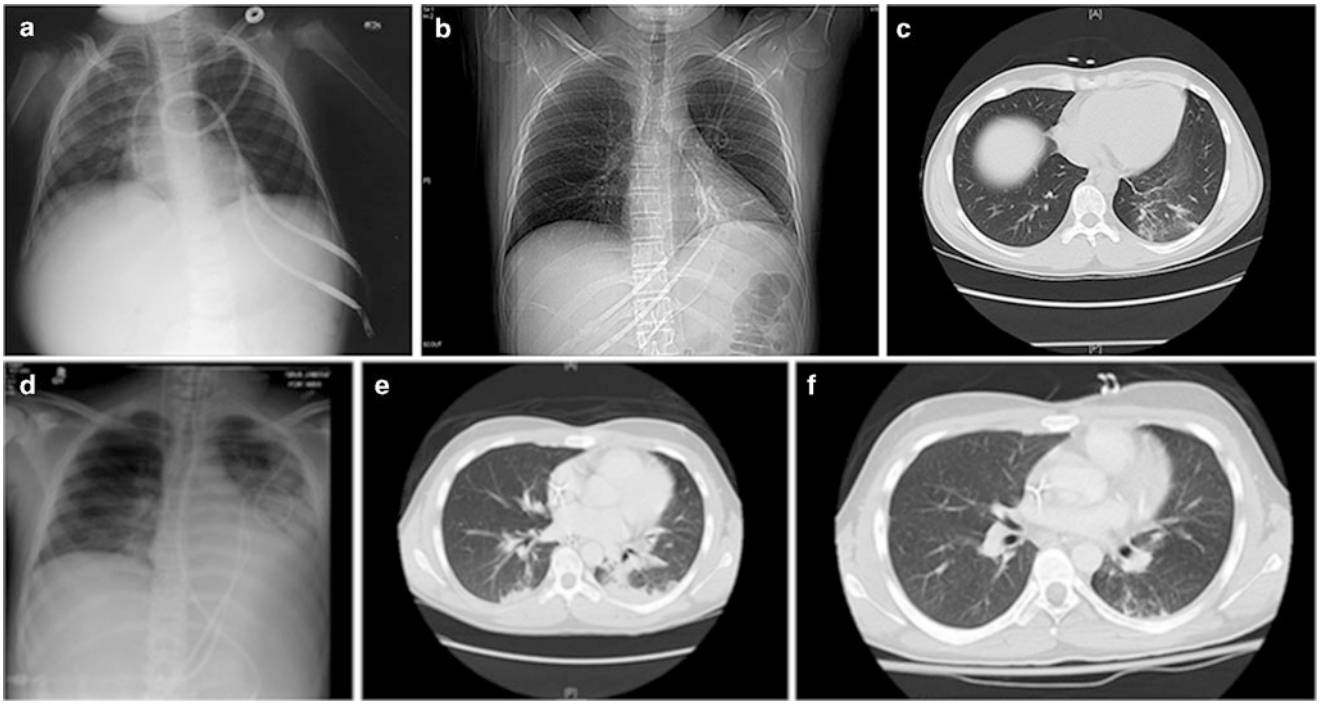

FIGURE 31-2. Chest radiographs of transplant recipients with RSV and hMPV lower respiratory tract disease. (**a**) Three-year-old with RSV pneumonia developing 1 week post HSCT, which proved to be fatal within 2 weeks despite antiviral therapy with aerosolized ribavirin. (**b**) HMPV Lower respiratory tract disease in a 16-year-old boy with ALL and hMPV disease diagnosed day 5 after a matched related BMT. He was diagnosed with hMPV infection on day #5 posttransplant, with clinical findings of mild respiratory distress with cough and abnormal chest radiograph showing interstitial infiltrates. Aerosolized ribavirin and IVIG therapy was initiated. (**c**) Posttransplant day #9: He required intubation due to respiratory failure and acute respiratory distress syndrome, with left sided pneumonia and increasing pleural effusion. He required mechanical ventilation and 50% FiO_2. (**d**) Post Tx day #11: Worsening of left lower basilar consolidation with small left pleural effusion, but oxygen requirement down to 35% FiO_2; not yet engrafted and continued on ribavirin. (**e**) Day #16: Now engrafted and ribavirin discontinued. He was able to be extubated and relatively quickly weaned to room air. (**f**) Patient with improvement post engraftment on day #16 posttransplant. Chest CT shows interval improvement in bilateral patchy groundglass opacities and in left lower lobe consolidation. He received supportive care and 10 days of aerosolized ribavirin therapy, which did not substantially impact viral load, but he improved clinically concomitant with engraftment. He was subsequently extubated and discharged several weeks later.

increased rates of hypoxemia [49]. Alveolar consolidation corresponds to more extensive damage on histological examination. Histological evaluation has also shown hyaline membrane formation, foci of bronchiolitis obliterans and organizing pneumonia and diffuse alveolar hemorrhage.

Variable disease severity has been reported following hMPV infection in SOT recipients. In one study of 114 lung transplant recipients, hMPV was detected in four symptomatic and one asymptomatic patients (4.3%), but viral infection was not persistent and resolved without major complications [88]. In another study, nine lung transplant recipients with hMPV were compared with 17 transplant recipients without hMPV infection; hMPV infection was associated with signs of acute graft rejection and increase overall mortality (three of nine with hMPV-infected patients died and none died in the hMPV-negative group) [54].

31.5.3 Outbreaks

Outbreaks of respiratory viruses occur annually in the community, with potential for patients, families, or health care workers to become infected following exposure outside the hospital. However, nosocomial transmission within the hospital setting becomes a serious concern because of the high rates of morbidity and mortality in immunocompromised patients documented in nosocomial outbreaks. Hospital-based oubreaks of RSV infection in HSCT recipients can occur through introduction of circulating community strains as well as transmission of identical viral strains among patients [42, 44]. Outbreaks of RSV have been associated with high mortality rates ranging up to 45% of infected patients [89, 90]. The transmission of identical strains of RSV within the outpatient setting into the hospital setting

has also been shown [91], demonstrating the importance of infection control measures in both the inpatient and outpatient setting. Nosocomial transmission of hMPV can also occur, with outbreaks possible in both inpatient and outpatient units. In one study, 15 patients were diagnosed with hMPV within 7 weeks in a tertiary care cancer unit [92]. Molecular subtyping revealed infection with genotype A2a virus, implicating nosocomial transmission. Four patients (26.6%) died from hMPV-associated pneumonia and consequent multi-organ failure.

31.6 Treatment and Prevention Strategies

31.6.1 RSV Treatment

Current options for the antiviral therapy of RSV disease in immunocompromised hosts are limited. Large, controlled, therapeutic trials for RSV pneumonia or lower tract disease in immunocompromised patients have not been conducted. Aerosolized ribavirin, licensed in the USA for the therapy of RSV bronchiolitis and pneumonia in infants and young children in 1986, is the antiviral agent currently utilized for the treatment of RSV disease in immunocompromised patients. In general, ribavirin is given as an inhaled aerosolized solution via a face mask in a protective environment, such as a "scavenging tent," to protect health care workers from potential drug contamination. Ribavirin was initially licensed for use when given for 12–18 h/day, but the use of intermittent aerosolized ribavirin given over 2 h, three times daily, was found to have similar effectiveness to 12–16 h/day continuous infusion ribavirin in healthy children [93–95] and has been utilized in adults because of ease of administration and enhanced tolerability. A randomized trial in HSCT patients at risk for LRTI evaluated intermittent dosing of ribavirin given over 2 h three times daily versus continuous ribavirin administration using an adaptive randomized trial design in 50 HSCT patients, with the authors concluding that the intermittent schedule was preferable because of ease of administration and evidence of higher efficacy [96].

Only one randomized, controlled, multicenter clinical trial of aerosolized ribavirin for the prevention of RSV disease progression to LRI has been conducted in HSCT recipients early posttransplant, and despite an enrollment period of several years, only 15 patients were enrolled [97]. None of the ten patients randomized to high dose, short duration aerosolized ribavirin (administered as 2 g/100 ml water given over 2 h three times daily) had disease progression compared to 2/5 control patients, a trend that was not statistically significant ($P=0.08$). Viral loads appeared to be reduced during the ribavirin treatment, but did rebound after cessation of therapy. Data demonstrating effectiveness of ribavirin is mainly retrospective. In an open trial in adult HSCT recipients with "RSV-induced acute lung injury," monotherapy with aerosolized ribavirin was reported to be of benefit if initiated prior to the development of radiographic infiltrates [6]. In another open trial in adult HSCT recipients with radiographically proven RSV pneumonia, combination therapy with aerosolized ribavirin and high RSV-titered IVIG was reported to be of benefit only if initiated prior to the onset of respiratory failure [8, 98]. A retrospective MDACC study of confirmed RSV infections in 280 allogeneic HSCT recipients from 1996 to 2009 utilized multivariable logistic regression to demonstrate that lack of ribavirin aerosol therapy at the upper respiratory tract disease stage was an important risk factor associated with RSV LRTI and all-cause mortality [99]. In a retrospective study of HSCT recipients with confirmed lower respiratory tract RSV infection based on analysis of bronchoalveolar lavage fluid at the Hutch, viral RNA detection in the blood was detected in 30% of 92 patients at a median of 2 days following diagnosis of lower respiratory tract disease [37]. Neutropenia, thrombocytopenia, and mechanical ventilation increased the risk of RSV RNA detection in the plasma or serum but lymphopenia and steroid use did not. The detection of RSV RNA in the serum or plasma increased the risk of overall mortality with an adjusted hazard ratio (AHR) of 2.09 ($P=0.02$).

Data in solid organ transplant recipients is even more limited. Favorable responses have been reported in an open trial of lung transplant recipients with lower respiratory tract disease who received monotherapy with aerosolized ribavirin [58], as well as open trials with oral ribavirin [100–102], although controlled studies have not been performed. Oral ribavirin was found to be well-tolerated, result in less hospitalization, and be less expensive than intravenous or inhaled ribavirin in a retrospective study of 52 lung transplant recipients [102].

The treatment of RSV disease with a combination of antiviral therapy and passively administered immunoglobulin has been investigated in animal models and in children [103–106]. Therapy with IVIG containing high levels of RSV-specific antibodies alone does not seem to be effective in placebo-controlled trials in children who were not immunocompromised [104, 107]. In small open trials at MDACC, combination therapy with aerosolized ribavirin (18 h/day) and high RSV-titered IVIG (0.5 g/kg every other day) was associated with a favorable response in adult HSCT recipients and patients undergoing induction chemotherapy for leukemia who had RSV lower respiratory tract disease in whom therapy was initiated prior to respiratory failure [8, 98]. At the Dana Farber Cancer Institute, combination therapy with aerosolized ribavirin (18 h/day) and RSV-IVIG (1.5 g/kg for one dose) was similarly associated with a favorable response in 2 HSCT recipients with clinically severe RSV pneumonia occurring early following transplant [105]. In subsequent years, MDACC has utilized a combined regimen with similar response, although standard IVIG in frequent and large doses (500 mg/kg QOD) has been substituted for high-titered IVIG.

Other therapeutic options for the treatment of RSV include the use of oral ribavirin, which has been studied in HSCT recipients and found to be safe and less expensive than intravenous or aerosolized ribavirin [100, 108, 109], and is recommended as an option in addition to intravenous and inhaled ribavirin by the European Conference on Infections in Leukemia [94]. Other options include IV ribavirin (an investigational drug, ICN) and topical immunoglobulins administered with aerosolized or intravenous ribavirin [105, 110, 111]. The relative ease of administration of IV ribavirin is attractive, but high rates of mortality (80%) and significant cases of hemolytic anemia (20%) make this option currently problematic. Although the European experience with combination aerosolized/intravenous ribavirin has been favorable [112] and intravenous ribavirin is relatively simple to administer, the high rates of mortality and significant cases of hemolytic anemia make this approach controversial. Monotherapy with IV ribavirin may be more toxic in these patients than has been previously reported in patients with hemorrhagic fevers [113, 114].

The decision to initiate therapy with aerosolized ribavirin with or without immunotherapy for a RSV-URI must take into consideration many factors, including the patient's risk of developing serious lower respiratory tract disease (and specifically, the degree of anticipated lymphopenia), the potential exposure of health care workers to the medication, the psychological and physical discomfort to the patients of aerosol therapy, the adverse effects of aerosolized ribavirin such as bronchospasm, the high cost of these drugs as well as the intensive respiratory therapy needed to safely administer aerosolized ribavirin, and the need for hospitalization with more frequent or prolonged ribavirin dosing regimens.

In patients who have already undergone conditioning therapy and stem cell infusion but have not yet engrafted, the initiation of antiviral therapy at the URI stage may be beneficial. Early studies conducted in the 1990s were small and uncontrolled. One study conducted at FHCRC treated 25 HSCT recipients with upper tract RSV disease with low dose aerosolized ribavirin administered at a high concentration (60 mg/ml) for 2 h each day (total: 2 g/day [8]). Unfortunately, 8/25 patients developed pneumonia, and seven of these died. Another study evaluated combination therapy with aerosolized ribavirin and 500 mg/kg IVIG every other day in 12 patients, two of whom developed pneumonia and died [8, 115]. This "preemptive" strategy, similar to that used in the prevention of CMV disease and CMV pneumonia, is used at some transplant centers for those patients at highest risk of RSV disease progression, such as pre-engrafted patients with RSV detected in the first weeks following transplantation. Other options for preemptive therapy include immunotherapy with IVIG or RSV-specific monoclonal antibodies, although little data on efficacy of immunotherapy is available.

31.6.2 HMPV Treatment

No antivirals for the therapy of hMPV are currently available or routinely utilized. Ribavirin is active in vitro and in vivo against hMPV, although there are no controlled studies or evidence from large retrospective reviews for the treatment of hMPV pneumonia in humans and no drug has yet demonstrated clinical effectiveness in humans [116, 117]. Intravenous, oral or inhaled ribavirin alone or in combination with IVIG has been reported as potentially successful therapeutic options. A retrospective analysis compared the outcome between 13 immunocompromised patients with hMPV pneumonia treated with ribavirin ± IVIG and ten untreated patients. Ribavirin treatment was associated with more hypoxemia and similar mortality, possibly related to late initiation of therapy [49]. A Seattle study describing hMPV lower respiratory tract disease in 55 immunocompromised children, including nine undergoing HSCT and eight SOT recipients, demonstrated that HSCT recipients had more evidence of severe disease [91]. Five of eight HSCT recipients but no SOT recipients had lower tract disease and were treated with aerosolized ribavirin; three had been diagnosed with hMPV pretransplant and during the posttransplant period received both ribavirin and IVIG. Two additional children received aerosolized ribavirin only. Ribavirin was generally administered at a dose of 2 g given three times daily for 5–11 days. Two of the three patients diagnosed with hMPV pretransplant who received ribavirin and IVIG died [91].

31.6.3 Infection Prevention Measures for RSV and hMPV

An aggressive infection control strategy can be effective in reducing the nosocomial acquisition of RSV by transplant recipients [91, 118]. Infection control strategies play a crucial role in the prevention of respiratory viral infection [89, 118–120]. An effective strategy is based on understanding the potential seriousness of these infections in transplant recipients, knowledge of the viruses circulating in the community, and ongoing surveillance in high-risk patients. Continuing education of patients, family members, visitors, and staff regarding the potential seriousness of these infections must be repeatedly emphasized. Frequent and routine clinical screening of high-risk patients for acute upper and/or lower respiratory tract illness or flu-like illness must be conducted, with sampling of respiratory secretions from symptomatic high-risk patients routinely performed both pretransplant and posttransplant. Each health care-acquired infection should be viewed as a sentinel event warranting an investigation and reaffirmation or modification of the preventative strategy.

Infection control strategies should be designed to prevent spread by multiple modes of transmission [90, 91]. Multiple

respiratory viruses may circulate in the community concurrently and can be spread by different means. Infection control measures may need to be intensified during community or hospital outbreaks, and the intensity and duration of infection control measures should be tailored to the risk of serious disease in different subsets of transplant recipients, and to what works in the "real world." Guidelines for the prevention of opportunistic infections among HSCT recipients have been issued by the Centers for Disease Control and Prevention (CDC), the Infectious Diseases Society of America, and European and American Societies for Blood and Marrow Transplantation. The guidelines clearly present evidence-based recommendations rated by the strength of the recommendation and the quality of the supporting evidence, similar to guidelines previously issued for the prevention of opportunistic infections in those with human immunodeficiency virus [120]. Preventive strategies for HSCT recipients, their household contacts and other close contacts, and health care workers are clearly outlined in this document.

The prevention of nosocomial acquisition of respiratory viral infections in HSCT recipients has been demonstrated in one prospective study comparing rates of infection in patients cared for in a "protected environment" with patients cared for on a transplant unit where infection control measures were strongly encouraged, but not rigidly enforced [9]. The effectiveness of infection control interventions has also been demonstrated by the dramatic decline in the frequency of nosocomial CRV infections among HSCT recipients cared for in this transplant unit after the implementation of an aggressive, multifaceted infection control strategy [118]. Although this intensive multifaceted approach has been effective, modified versions of this strategy have also been effective. For instance, the Seattle Cancer Care Alliance (SCCA) adult inpatient transplant unit uses a similar strategy with the exception that health care workers and other visitors do not wear masks when entering the patient room [91]. However, these workers are intensively screened for signs and symptoms of respiratory illnesses prior to entering the unit, and restricted from entering the unit if they are symptomatic. Similarly, HSCT recipients with respiratory symptoms are not transferred to other units, but are cared for on the transplant unit using modified droplet precautions with all persons entering the room wear gloves, gown, and mask, and the door to the room is kept closed. It may not be feasible to so intensively protect patients for the duration of increased susceptibility to respiratory viral diseases. Protective strategies are costly and cumbersome, and pose unpleasant restrictions on the freedom and quality of life of the patient and their families. This problem is further compounded by the growing trend to discharge patients early from the hospital and to perform outpatient HSCT or posttransplant care. Transplant recipients residing in the community and followed frequently in the outpatient setting are another group in which infection control practices must become priority [91].

The prevention of exposure to respiratory viruses is particularly challenging among high-risk transplant recipients living in the community because respiratory infections are so prevalent and so contagious. Examples of protective measures for outpatients include washing hands frequently and thoroughly, avoiding close contact with individuals suffering from respiratory illnesses, and encouraging close contacts to vigorously practice respiratory hygiene. In many cases, such as individuals living with children, such efforts may be nearly impossible. Consideration of removing day care exposures for young children or decreasing exposure to transplant recipients to children (including siblings), can and should be discussed with families. The rigor and duration of prophylactic measures need to be individualized based on the immunologic status of the patient and the risk for serious disease, the needs of the patient, and quality-of-life issues.

31.6.4 Passive Immunoprophylaxis

Passive immunization with immunoglobulin, immunoglobulin products, and humanized monoclonal antibodies have been actively studied in the pediatric infant population. Palivizumab, a humanized monoclonal antibody directed against the RSV F protein, is licensed for the prevention of RSV disease in premature infants and infants with congenital heart disease, and is administered as a monthly injection during the 4–5 months of RSV season. The cost of this therapy has led to new guidelines for use in the pediatric population [121]. Similar interventions have been utilized to attempt to decrease the morbidity of serious RSV disease in HSCT recipients but direct proof of effectiveness has not yet been demonstrated [115]. For example, passive immunoprophylaxis in immunocompromised patients has been evaluated in an open trial conducted [122] using high-titered human RSVIG. In this adult study, significant antibody titer increases to other respiratory viruses were extremely variable, although the subset of patients with the lowest titers appeared to receive the greatest increase in viral-specific antibody. The cost of this potential therapy remains quite high. The monoclonal RSV antibody palivizumab (Synagis) has been studied in an open label study in adult HSCT recipients [123]. Immunoprophylaxis with monoclonal RSV antibodies would be prohibitively expensive in older children and adults, but may have the potential to protect against RSV infection, based on pediatric studies.

New antibody products, including long-lasting monoclonal antibodies with enhanced activity against RSV, are under development and if available at a less expensive price, could potentially provide protection against RSV for patients in the pretransplant and immediate posttransplant phase. Newer monoclonal antibodies that have the potential to neutralize against both RSV and hMPV have also been described, offering hope for newer preventive modalities that may protect against both these viruses [124].

31.6.5 Pretransplant Screening

Screening of transplant recipients for respiratory viruses prior to transplant is not routinely recommended based on current US and international transplant guidelines [94, 125]. However, the assessment of viral shedding or infection in symptomatic transplant candidates prior to transplant is recommended. Delay of transplant based on detection of RSV or hMPV may be warranted depending on the evaluation of the risk and benefits of continuing on with transplantation. Consequences of postponing transplantation should be considered, including progression of underlying malignancy, logistical issues regarding the donor availability, and accessibility of services for the patient. An early study of RSV diagnosed prior to HSCT demonstrated that delaying transplant reduced the risk of pneumonia following transplant [23]. More recently, a large prospective study conducted in 458 patients at the Hutch demonstrated that nearly 25% of subjects had respiratory viruses detected pretransplant [24]. Overall, patients with a virus detected prior to transplant had fewer days alive and lower survival at day 100 (AHR 2.4; 95% CI 1.3–4.5) compared with patients who did not have viruses. In HSCT recipients who had respiratory symptoms and a virus detected prior to transplant (mainly adults), an increased overall mortality was seen compared with patients without respiratory viruses detected. Higher rates of pretransplant infection and sequelae of infection were seen in pediatric patients. Detection of respiratory viruses in asymptomatic patients was not associated with increased mortality. This data strengthens current guidelines that recommend patients with respiratory symptoms prior to transplant should be tested for respiratory viruses and transplant delayed, when feasible. However, this study was performed chiefly in adults. The higher rates of respiratory viruses documented in children pretransplant make routine screening of pediatric patients worthy of further investigation.

31.6.6 New Antivirals

Experimental approaches to the therapy of RSV antiviral therapy include novel fusion inhibitors [126], nucleoside agents, small RNA inhibitory molecules [109], and high-titered monoclonal antibody preparations. Two promising RSV antivirals that have shown efficacy in challenge studies in healthy adults include the Alios compound AL8176 (Alios Biopharma, South San Francisco, CA), an oral anti-RSV nucleoside designed to inhibit RSV replication by acting on the viral polymerase, and the Gilead Sciences compound GS5806 (Gilead Sciences, Foster City, CA), an orally bioavailable RSV fusion inhibitor [126, 127]. Clinical trials of these agents in healthy young children with RSV have been proposed [128]. An international multicenter, placebo-controlled clinical trial of GS5806 was initiated in July 2014, and remains ongoing in adult HSCT recipients with documented RSV infections (Clinicaltrials.gov identifier NCT02135614). Other antiviral agents are under development.

31.6.7 Vaccines

No RSV or hMPV vaccine is currently available. In general, active immunization of transplant recipients will be unlikely to prevent severe disease seen in the first several months posttransplant. However, prevention of RSV infection in families, staff, and nosocomial spread of virus by the use of vaccines holds true promise to benefit transplant recipients themselves. Promising advances in new vaccines directed against both RSV and hMPV have been reported over the past decade, with progress evident in both RSV fusion-protein based vaccines and live attenuated vaccines. Advances in the understanding of the pre- and post-fusion nature of the RSV F protein [129] has led to increased work in developing protein-based vaccines appropriate for older children, adults, and pregnant women.

Advances in technology and better molecular understanding of RSV and hMPV have resulted in new potential RSV candidate vaccines [130]. At least 12 RSV vaccines are in clinical studies in phase 1 or 2 clinical studies, with one RSV F vaccine under study in pregnant women. Examples of live RSV vaccine candidates under study include live-attenuated vaccines relying on genetic manipulation of the RSV genome [131], vectored virus vaccines utilizing the chimpanzee adenovirus or vaccinia virus Ankara [132], or chimeric viruses containing a backbone of attenuated parainfluenza with the F gene of RSV added [133, 134]. A chimeric hMPV vaccine containing a backbone of avian hMPV and F genes of the hMPV [135]. Although live viral vaccines are unlikely to be given to transplant recipients pretransplant or early posttransplant, they offer hope for the potential control of RSV and hMPV disease in family members and health care workers the future.

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32 Rhinovirus, Coronavirus, Enterovirus, and Bocavirus After Hematopoietic Cell Transplantation or Solid Organ Transplantation

Alpana Waghmare and Michael Boeckh

32.1 Rhinoviruses

32.1.1 Epidemiology

Human rhinoviruses (HRVs), the viruses predominantly associated with the common cold, are highly prevalent in both immunocompetent and immunocompromised individuals. Prior to the development of sensitive molecular viral detection assays, influenza, respiratory syncytial virus, and parainfluenza virus were the most common and most concerning respiratory viral pathogens detected in hematopoietic cell transplant (HCT) recipients [1]. Due to the development of polymerase chain reaction (PCR) assays for viral detection, HRVs are now known to be the most common viruses detected from respiratory specimens in HCT recipients and can account for 25–40% of cases of viral respiratory infections in these patients [2–4] (Figure 32-1). Due to their high prevalence and their ability to cause progressive infection, HRVs are also a significant cause of lower respiratory tract infection (LRTI) in HCT recipients (Table 32-1). HRV infection is also common in solid organ transplant (SOT) recipients, although the incidence is not known among SOT recipients as a whole. In lung transplant recipients, data from older retrospective and prospective studies suggests an incidence of 35–55% among patients with positive respiratory samples [5–7] (Figure 32-2). In a recent prospective surveillance study of 112 lung transplant recipients, HRVs represented 62% of all positive samples [8]. Among symptomatic lung transplant recipients, HRV represented 34% of all respiratory viruses detected [9].

HRVs are members of the Picornaviridae family and are classified into three species, HRV-A, HRV-B, and HRV-C, based on similarity in genome organization, capsid features, and conserved sequences [10]. The total number of genotypes continues to grow as new genotypes are characterized; currently at least 160 unique genotypes are described. Due to poor growth in traditional viral culture models, HRV-C was only recognized after the development of molecular diagnostic techniques. Thus, HRV-C is not a novel species, but rather one that has been circulating unnoticed due to lack of an appropriate diagnostic assay. There are several biologic characteristics of HRV-C that differentiate the species from HRV-A and HRV-B. HRV-A and HRV-B both use ICAM-1 or LDLR for cell attachment and entry, whereas it appears that HRV-C may utilize a distinct receptor, cadherin-related family member 3, that is associated with asthma susceptibility [11, 12]. Additionally, HRV-C species are stable at higher temperatures and readily infect upper and lower airways, whereas HRV-A and HRV-B species tend to be more limited to the sinuses and upper airways [13, 14]. These biologic characteristics are thought to play a role in variations in clinical outcomes observed among the different species.

32.1.2 Clinical Characteristics

Most immunocompetent patients with HRV present with an afebrile, self-limited syndrome characterized by rhinorrhea, nasal congestion, and malaise, and less frequently sore throat, mild cough, and hoarseness [15–19]. HRV may also be associated with exacerbations of sinusitis, chronic bronchitis, and asthma, and with lower respiratory tract syndromes and atypical pneumonias in otherwise healthy people, including the young and the elderly [20, 21]. The specific mechanisms by which HRVs produce lung diseases are not well understood. HRVs are also implicated in asthma and chronic obstructive pulmonary disease (COPD) exacerbations, but again the mechanisms are poorly defined.

With the widespread availability of PCR diagnostics, data are emerging on the incidence and clinical relevance of HRV infections in immunocompromised patients. Early studies relied on culture to detect HRV, a specific but insensitive method because the standard viral culture systems are not optimized for HRV detection, especially HRV-C [22]. For example, a Fred Hutchinson Cancer Center surveillance study from 1987 to 1992 detected HRVs in 29 specimens, and only one was from a lower respiratory tract specimen [2]. A prospective 5-year study at MD Anderson Cancer

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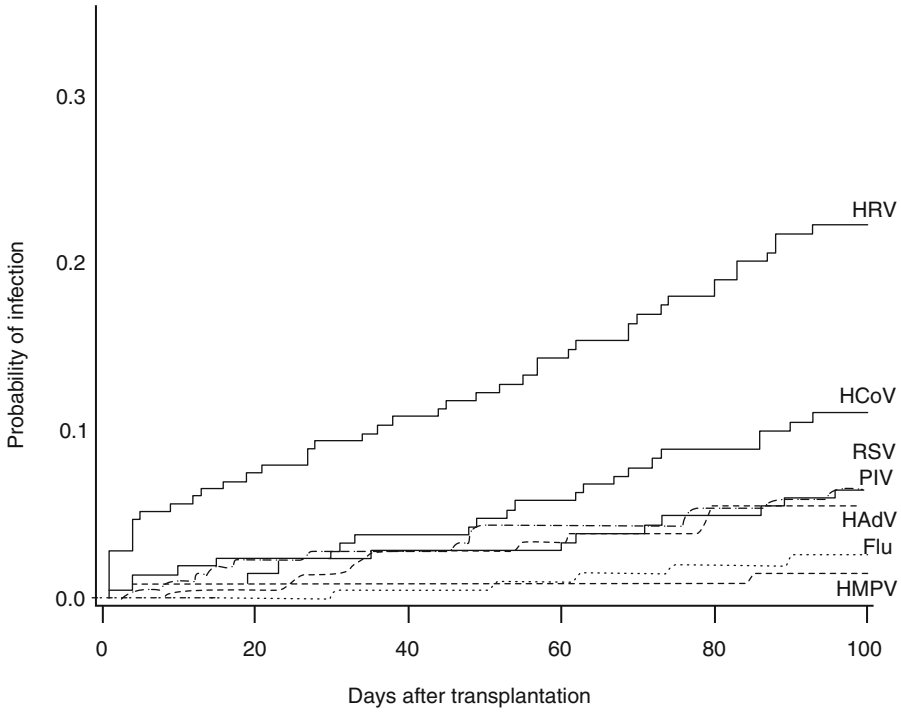

FIGURE 32-1. Cumulative incidence of first infection episodes of HRV, HCoV, and other respiratory viruses (RSV, PIV, HMPV, influenza, adenovirus) after transplantation in 215 HCT recipients. Reproduced from Blood, Human rhinovirus and coronavirus detection among allogeneic hematopoietic stem cell transplantation recipients, Filippo Milano, Angela P. Campbell, Katherine A. Guthrie, Jane Kuypers, Janet A. Englund, et al., 115 (10): 2088–2094, 2010, with permission from Springer Science and Business Media.

TABLE 32-1. Summary of clinical manifestations of rhinoviruses, coronaviruses, enteroviruses, and bocavirus

Virus	Upper respiratory tract infection	Lower respiratory tract infection	Other manifestations	Treatment	Comments
Human rhinovirus	++++	++		Supportive care	May be associated with severe disease in immunocompromised hosts
Coronavirus	+++	+	Gastrointestinal disease in children	Supportive care	Recent outbreaks of severe acute respiratory syndrome (SARS) and middle east respiratory syndrome (MERS)
Enterovirus	++	+	Neurologic disease (poliomyelitis, meningitis, encephalitis), cardiac disease, muscle disease, eye infections	Supportive care	Sporadic outbreaks described, including Enterovirus-D68
Bocavirus	++	?		Supportive care	

Center cultured specimens specifically for HRVs at lower temperatures with roller culture methods, and reported that HRV infections were associated with substantial morbidity and mortality in 7 of 22 (32%) myelosuppressed patients [23]. In that study, approximately one third of the adult HCT recipients who developed symptomatic HRV infections prior to engraftment had progression of upper respiratory tract symptoms to LRTI, and all cases with pneumonias were fatal. Lung biopsies and autopsies revealed findings consistent with interstitial pneumonitis and/or ARDS, but no in situ evaluation was performed to definitively assess HRV infection. Similar reports with evidence of LRTI based on radiographic and BAL findings continue to be noted [24–26], but it remains unknown if pneumonia is a direct cause of viral invasion of the lung tissue or by host responses in the lung. Evidence for in vitro and in vivo replication in lower respiratory tract has been shown in experimental infection, where HRV was isolated from human volunteers after intranasal HRV challenge by in situ hybridization [27]. The use of RT-PCR continues to provide new information about the frequency of HRV infection. In a study of BAL samples from 77 HCT recipients that were tested using RT-PCR, HRV was detected in six patients (8%), mortality rate was very high (83%) and two of the six patients showed persistent HRV infection. However, all of the HRV-infected patients had significant coinfections and it was not certain whether HRV

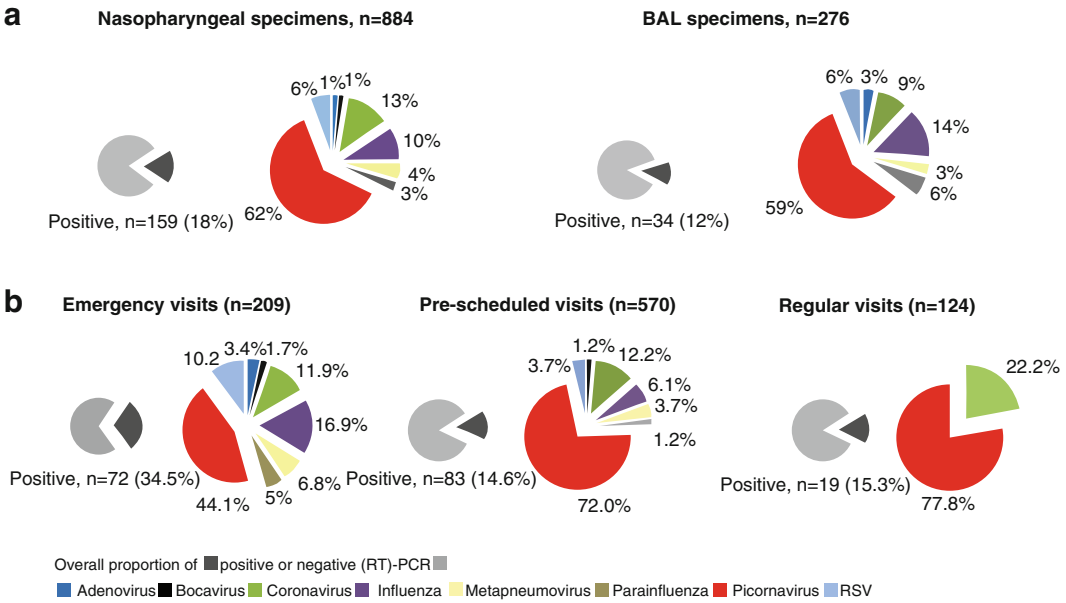

FIGURE 32-2. Distribution of viruses in lung transplant recipients. (**a**) Viruses recovered in upper and lower respiratory specimens. (**b**) Viruses recovered according to visit type. Reproduced from Thorax, Pierre Olivier Bridevaux, J.D. Aubert, P. M. Soccal, J. MazzaStalder, C. Berutto, Incidence and outcomes of respiratory viral infections in lung transplant recipients: A prospective study, 69, pages 32–38, copyright 2014, with permission from BMJ Publishing Group Ltd.

infection was the direct cause of poor prognosis [25]. In a small cohort of patients with hematologic malignancy, LRTI was associated with hypoalbuminemia and bacterial co-pathogens were seen in 25% of patients [28].

Recent studies have shown that immunocompromised adults with HRV demonstrated similar hospital admission rates, intensive care unit admissions, and mortality rates as patients with pandemic H1N1 influenza [29]. Several reports have linked HRV infection to severe respiratory failure and even death [23–25]. Furthermore, recently presented data suggest that LRTI associated with HRV leads to a mortality rate comparable to that of RSV, influenza virus, and PIV [30], independent of the presence of co-pathogens. Risk factors for mortality following HRV LRTI included bone marrow stem cell source, oxygen requirement at time of diagnosis, and steroid use ≥1 mg/kg prior to diagnosis [30]. Other factors that may influence clinical severity include the presence of HRV RNA in blood, viral load, and HRV species type; however, no data exist in immunocompromised patients to date. HRV viral RNA was detected in the sera of 30 (12%) of 243 pediatric patients with severe HRV respiratory infection, with HRV-C being the predominant species [31]. In healthy pediatric patients, increased respiratory viral load has been associated with HRV LRTI and HRVC has been implicated as a more virulent pathogen [32–34]. Others, however, have shown lack of correlation between HRV-C and oxygen requirement, length of hospitalizations, and coinfections [35]. The predominance of HRV-C in HCT recipients has also been described in small studies, with higher rates of pneumonia in patients with HRV-C detected from the upper respiratory tract [36]. In a small cohort of patients with hematologic malignancies, the rate of LRTI was not different between patients infected with HRV-A, HRV-B, or HRV-C [28]. The relative risk of HRV-C infection in the immunocompromised population remains unknown, and more research is needed to define the role of strain differences on outcomes.

Detection and diagnosis of respiratory viral infections prior to transplant is a common clinical concern that has until recently only been evaluated in small cohorts for certain viruses [37–40]. In a large, prospective surveillance cohort of allogeneic HCT recipients, detection of HRV pretransplant was associated with significantly fewer days alive and out of the hospital, and significantly higher mortality at 100 days posttransplant [41]. Further, larger prospective studies are needed to determine risk factors for posttransplant complications, the role of viral load and symptom burden at the time of transplantation, and the need to potentially delay transplantation for patients with HRV present prior to transplantation. Ultimately, the issue of viral causality of disease and evaluation of prophylactic and treatment modalities will need to be addressed. The impact of HRV infection prior to SOT is not known.

Like HCT recipients, SOT recipients are exposed to highly immunosuppressive regimens that leave them susceptible to respiratory viral infections. Lung transplant recipients have the added disadvantage of altered lung immunity due to factors such as impaired ciliary clearance, poor cough reflex, and abnormal lymphatic drainage. These factors can predispose to lower respiratory tract infections. The impact of HRV on outcomes in lung transplant recipients can range from

asymptomatic infection to severe disease. In a pooled analysis of all respiratory viruses detected in lung transplant recipients, viruses were detected five times more frequently when respiratory symptoms were present [42]. A correlation between higher symptom scores and higher rhinovirus load in the upper respiratory tract has been demonstrated, although even asymptomatic patients can have relatively high viral loads [43]. The relative rate of progression from upper to lower tract disease for HRV specifically is not known, although the effect on lung function has been evaluated in aggregate for all respiratory viruses and suggests a decline in forced expiratory volume (FEV1) of −5% to −30% [42]. For HRV specifically, the FEV1 loss was similar to that seen in other respiratory viruses [8]. The correlation between respiratory viral infections and acute rejection, chronic rejection, and bronchiolitis obliterans syndrome (BOS) remains somewhat unclear, with several conflicting findings when respiratory viruses were evaluated in aggregate [6–8]. A recent large cohort of 250 lung transplant recipients, however, showed an independent association between respiratory viral infections (34% HRV) and chronic lung allograft dysfunction in multivariate models [9]. This association was influenced by time, with more of an effect within a shorter period following respiratory infection. Larger, prospective studies investing individual viruses are needed to clearly assess the impact on these outcomes.

32.1.3 Diagnosis

Unlike paramyxoviruses, HRV infection cannot be diagnosed based on characteristic histopathologic changes or changes in cell morphology. In the past, cell culture was used to diagnose HRV infection using multiple cell lines at low temperatures of 33–34 °C, often in rolling tubes. The cell lines utilized for the detection of HRVs may detect enteroviruses; HRV isolates are distinguished from enteroviruses by their lability in acid (loss in viral titer following exposure to a pH of 5). There are no commercially available antigen-detection assays or simple kits for the detection of HRV.

RT-PCR has dramatically improved the ability to both detect and characterize HRVs, with current assays at least two to three times more sensitive than conventional culture methods [44]. Some PCR assays are able to distinguish between enteroviruses and HRVs instead of the acid lability assays [45]. Typing of HRVs based on PCR amplification sequence variations in 5′-noncoding region also has been described [46]. New standardized methods to detect more of the over 100 strains of HRV have now been described [47]; however, commercially available multiplex respiratory viral PCR panels contain primer/probe sets that can cross-react between enterovirus and HRV strains. New strains and types of HRV are being detected frequently and more diseases associated with HRV are being described using new and diverse molecular methods.

32.1.4 Treatment Options

There are no approved antivirals for the treatment of HRV infections. Several agents have been evaluated in preclinical and clinical trials for the treatment of HRV infection in immunocompetent hosts, including capsid binding inhibitors, protease inhibitors, and RNA synthesis inhibitors [48]. None of these agents have been evaluated in immunocompromised hosts. Given the high prevalence and potential severity of HRV infection in this population, there is a great need for drug development and clinical trials for the prevention and treatment of LRTI. Outside of transplant recipients, there is a potential need for intervention in other populations such as patients with asthma or COPD to prevent disease exacerbation [49, 50].

32.2 Coronaviruses

32.2.1 Epidemiology

CoVs are a frequent cause of the common cold, but little is known about the role of CoVs in immunocompromised patients [51] (Table 32-1). Human group 1 (subtypes 229E and NL63) and human group 2 (OC43 and HKU1) CoVs were originally reported as causes of human respiratory illnesses. The availability of more sophisticated diagnostic tools, such as RT-PCR, has facilitated the detection of CoVs in normal and immunocompromised persons. These improved molecular methods of viral discovery facilitated the recent identification of the novel Group 1 and 2 human CoV subtypes—NL63 in 2004 [52] and HKU1 in 2005 [53]. A more accurate clinical epidemiology of CoV infection is beginning to emerge. It is now known that all four known subtypes of CoV circulate simultaneously [54], and that in addition to the common cold, CoV is associated with upper respiratory tract infection and LRTI in persons with and without underlying conditions [55, 56]. In lung transplant recipients, CoVs appear to be the second most common respiratory viruses after picornaviruses with a detection rate of 13–27% of positive samples [5–7] (Figure 32-2). In a prospective surveillance cohort of lung transplant recipients, coronaviruses were detected in 13% of all positive samples, again only second to picornaviruses [8].

Two additional CoVs associated with outbreaks are the severe acute respiratory syndrome-associated CoV (SARS-CoV) and the recently described Middle East respiratory syndrome-CoV (MERS-CoV). The SARS outbreak originated in Guangdong Province in China in 2002 and was characterized by a life-threatening, atypical pneumonia and was spread by close contact with infected humans, mostly to household contacts and health care workers [57]. SARS-CoV is not currently circulating in the world with the most recent human cases of infection reported in China in 2004 [58]. MERS-CoV first emerged in the Arabian Peninsula in 2012, and since then travel-associated cases have been found

in a number of countries outside the region [59]. In adults, the fatality rate is estimated to be 40%; in children asymptomatic infection is common but patients with underlying medical conditions are at increased risk [60, 61]. There is little data on the incidence of SARS-CoV and MERS-CoV in immunocompromised hosts, although immune suppression is considered a risk factor. SARS-CoV has been described in liver transplant recipients and in patients with myelodysplastic syndrome [62, 63]. MERS-CoV has been described in patients on chronic immunosuppression and in renal transplant recipients with a broad range of clinical presentations [64, 65]. Other CoVs have been reported to cause pneumonia in children and immunocompromised patients treated for hematologic malignancies [66–68]. The role of coronavirus virus infection prior to transplantation is not known.

32.2.2 Clinical Characteristics

Although most CoV infections result in relatively mild upper respiratory tract infection, these viruses have been associated with more severe LRTI (e.g., bronchiolitis and pneumonia) in patients who are immunosuppressed, have asthma, or are premature. In one retrospective study carried out over 1 year, CoV was detected in six immunocompromised children—five with acute lymphocytic leukemia and one renal transplant recipient [54]. Five patients were febrile at the time coronavirus was present, with fevers lasting 1–7 days. All patients initially presented with rhinorrhea and nasal discharge; two children had cough as a presenting symptom. Chest radiographs of only one of the three children were abnormal; LRTI based on decreased oxygen saturation, tachypnea, and abnormal chest radiograph was present in only one child with leukemia, who was significantly neutropenic and lymphopenic at the time CoV was detected. CoVs have been associated with LRTI in HCT recipients with sometimes fatal outcomes [66, 67, 69–71]. The clinical characteristics of SARS-CoV and MERS-CoV infection in HCT and SOT patients are not well described, and presentation can range from mild symptoms to respiratory failure and death [62–65, 72].

32.2.3 Diagnosis

Until the advent of RT-PCR, techniques for the detection of CoV were limited and the reliable identification of CoV was problematic. Early detection techniques isolated two subtypes—OC43 and 229E, originally using organ cultures of human embryonic trachea, with morphology determined using negative staining with electron microscopy [73]. With the advent of molecular detection methods and increased interest in CoV detection during the SARS outbreak, new strains of CoVs have been discovered and new RT-PCR assays developed that facilitate further studies of these viruses. Based on RT-PCR assays, four strains of non-SARS CoVs (OC43, 229E, NL63, and HKU1) appear to cocirculate during the non-summer months in temperate climates, and are associated with symptomatic disease in immunocompromised hosts [54]. Guidance on RT-PCR and serologic assays for the confirmation on MERS-CoV can be found on the World Health Organization website [74].

32.2.4 Treatment Options

There are no approved antivirals for prophylaxis or treatment of CoV infections and supportive care remains paramount in managing patients infected with coronaviruses. Though several antivirals were used during the SARS-CoV epidemic, no clear benefit could be established on systematic review [75]. Oral ribavirin was evaluated in retrospective studies for the treatment of MERS-CoV in immunocompetent individuals; decreased survival was noted in one study when compared to matched controls [76, 77], however, larger prospective studies are needed to show true efficacy. Shedding of all coronaviruses may persist for up to months, and routine infection control practices are encouraged.

32.3 Enteroviruses

32.3.1 Epidemiology

EVs are part of the picornaviridae family of viruses and can be associated with severe illness in immunocompromised hosts. EVs include polioviruses, coxsackieviruses, and echoviruses; these are now all classified into four species: *Enterovirus A* (EV-A), EV-B, EV-C, and EV-D. Risk for infection and subsequent poor outcomes appears to be heavily influenced by age, although factors such as sex and socioeconomic status play a role in the general population. EV activity can be either sporadic or epidemic, and several outbreaks have been described. EVs are typically found during the summer and early autumn in temperate climates.

Enterovirus-D68 (EV-D68) was first identified in California in 1962 [78] and has since been associated with several small outbreaks, both in the US and internationally, from 2009 to 2013 [79–84]. In the summer of 2014, several hundred cases of severe respiratory illnesses in children in the United States were found to be associated with EV-D68 infection [85], and several additional clusters have been described worldwide [86–96].

32.3.2 Clinical Characteristics

EVs can cause a wide spectrum of illnesses in immunocompetent individuals including asymptomatic infection, poliomyelitis, meningitis, encephalitis, cardiac disease, muscle disease, eye infections, respiratory infections, exanthems, and neonatal disease. The most frequently described manifestation

in immunocompromised patients is respiratory disease, although the incidence and spectrum of disease is not known. According to one study, EVs can be associated with lower respiratory tract infection and mortality; however, larger studies are needed to establish specific risk factors for worse outcomes [97].

Most confirmed cases of EV-D68 infection have been in children, occurring primarily in patients with underlying lung disease such as asthma or a history of wheezing. EV-D68 was also associated with several cases of acute flaccid paralysis in children during the 2014 outbreak in the United States, although definitive causation has not yet been established [98, 99]. The impact of EV-D68 infection in immunocompromised hosts is not known; however, the association between EV-D68 and severe illness was described in eight adult immunocompromised patients with presumptive EV-D68 infection including HCT recipients [100]. Additionally, one recent report of adults with confirmed EV-D68 infection included solid organ transplant recipients [87].

32.3.3 Diagnosis

Depending on the clinical scenario, EVs can be detected from a number of clinical specimens including cerebral spinal fluid, serum, respiratory specimens, cardiac tissue, and stool. EVs may be identified in throat samples as well as fecal specimens and cerebrospinal fluid. Commercial multiplex PCR assays contain primer/probe sets that may cross react between rhinoviruses and enteroviruses. A specific EV-D68 RT-PCR has been developed by the CDC and has been made publically available [101].

32.3.4 Treatment Options

There are no approved antivirals approved for the treatment of EVs. Intravenous immunoglobulin (IVIG) has been used in the treatment of neonatal enteroviral sepsis, but the effect on clinical outcomes is highly dependent on the presence of specific neutralizing antibodies and timing of administration [102, 103]. Pleconaril, an oral capsid inhibitor with activity against picornaviruses, has been evaluated in treatment of enteroviral infections including meningitis, neonatal sepsis, and respiratory infections [104–108] but is not available for treatment. Other capsid binders, protease inhibitors, and polymerase inhibitors are in various stages of development, but none are currently available for treatment of enteroviral infections [109]. No studies have shown efficacy in immunocompromised hosts.

32.4 Bocavirus

Human bocavirus (HBoV) is a newly identified human parvovirus that was originally identified by random PCR amplification/cloning technique on pooled respiratory secretions from hospitalized children with respiratory tract symptoms [110]. This virus was named "human bocavirus," due to its relatedness to the genome organization of two other parvoviruses, bovine parvovirus and minute virus of canines, in the family Parvoviridae. This virus continues to be detected in young children with a winter seasonality [111–113]. The relationship of HBoV and respiratory disease in immunocompromised patients is not yet clear. Preliminary evidence to date demonstrates case reports of disseminated HBoV infection with involvement of the respiratory tract, blood, and stool in several patients, sometimes associated with GVHD and prolonged viral shedding in the feces [114, 115]. Other studies have reported little evidence linking this virus with pulmonary pathology or severe respiratory disease in HCT or lung transplant recipients [116–118]. Further research is necessary to link this virus with the disease in the transplant recipient. No specific antiviral therapy is available.

32.5 Future Directions and Unmet Needs

Respiratory viruses are a significant concern following HCT and SOT and can be associated with substantial morbidity and mortality, even among viruses traditionally not concerned pathogenic. New, sensitive diagnostic assays allow for routine detection of rhinoviruses, enteroviruses, coronaviruses, and bocavirus, and additional data on the epidemiology, risk factors, outcomes of infection, and the impact of different viral strains are desperately needed. Preliminary studies suggest that detection of these viruses prior to transplant may affect outcomes, but additional studies are needed to explore this important clinical area. Furthermore, as new antivirals are being developed, it will be important to identify high-risk patients that may benefit from treatment. Finally, a better understanding of these viruses will be able to inform better infection prevention strategies that will remain the mainstay of viral control.

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33
Adenovirus Infection in Allogeneic Stem Cell Transplantation

Susanne Matthes-Martin

33.1 Introduction

Adenoviruses (HAdVs) are nonenveloped lytic DNA viruses capable of infecting most mammalian species. To date 67 different human serotypes with varying tissue tropism have been identified, which are divided into seven subgroups or species (A–G) according to their oncogenic potential in rats, hemagglutinating properties, and DNA homologies [1–6].

About half of the serotypes have been associated with clinical symptoms, others are found rarely and may not cause disease [7]. HAdV is highly resistant even in moisture-free environments and remains infectious at room temperature for up to 3 weeks [7, 8]. HAdV is stable at low pH and resistant to gastric and biliary secretions, allowing the virus to replicate and achieve a high viral load in the gut [7].

HAdV is transmitted from person to person by viral shedding in feces, respiratory secretions, and tears of infected individuals. Infection occurs by receptor-mediated endocytosis. Receptors interacting with HAdV include the coxsackieadenovirus receptor, CD46, sialic acid residues, and lactoferrin [9, 10]. Upon entering early endosomes the virus particles gain access to the nucleus, and viral proteins enter the MHC class I and II processing pathways. Finally the replication of viral DNA leads to viral assembly, host cell lysis, and escape from the host cell [9].

Due to the fact that recombinant adenoviruses are increasingly being used as gene transfer vectors in humans, innate and adaptive immune responses to HAdV have been extensively studied. HAdV is recognized through intracellular and extracellular receptors, which trigger interferon-γ (INF-γ), tumor necrosis factor (TNF), interleukin-1 (IL-1), IL-2, and macrophage inflammatory protein production. Besides the direct antiviral effect of these cytokines by inhibiting assembly and maturation of adenoviral particles, they recruit and activate innate effector cells [11–14]. Finally HAdV-specific cytotoxic T-cell clones (mostly $CD4M^+$ cells) are able to lyse infected target cells using a perforin-dependent mechanism [15–17]. One of the most important T-cell targets is the adenoviral hexon protein, which contains the generic antigenic component common to all adenoviruses [18]. Leen et al. characterized 40 CD4- or CD8-restricted hexon epitopes and showed that most of them are shared among different HAdV subspecies suggesting that HAdV-specific T cells can recognize different serotypes [19, 20]. It is supposed that the exposure to different species during childhood and the resulting crossreactive cytotoxic T lymphocytes (CTLs) leads to a broad HAdV immunity in adults [20–23]. By cytokine flow cytometry and INF-γ ELSPOT HAdV-specific T cells are detectable in healthy individuals [24].

However, HAdV disposes of several mechanisms for immune escape. HAdV encodes proteins that block responses to cytolytic and proinflammatory cytokines, intrinsic cellular apoptosis, as well as innate and adaptive cellular immune responses [25–28]. Moreover, antigen presentation by MHC class I molecules is inhibited by blocking their transport to the cell surface [29].

Clinical manifestations in immunocompetent hosts include upper respiratory disease (subgroups C and E), gastroenteritis (subgroups A, D, and F), or conjunctivitis (subgroup D) and are self-limited in most cases although severe manifestations including encephalitis, myocarditis, and pneumonia have been observed sporadically [30–33]. HAdV infections occur mainly in young children; however, outbreaks of HAdV infections in adults, in military camps and in medical facilities have been described [31, 34–37]. In a US population-based epidemiological study, Gray et al. identified several risk factors for severe HAdV disease: age less than 7 years, chronic disease, recent transplantation, and infection with type 5 (subgroup C) and type 21 (subgroup B) [38]. Asymptomatic HAdV infection can persist lifelong in lymphoepithelial tissues [39].

Sites of HAdV persistence include tonsillar and adenoidal T-lymphocytes, and the gastrointestinal tract [39–41]. Diagnostic approaches include virus isolation from either conventional or rapid cell culture, serologic tests, direct fluorescent assay or enzyme immunoassay, and conventional or

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real-time polymerase chain reaction (PCR) techniques [5, 6, 42, 43]. Whereas conventional cell culture is relatively time-consuming, enzyme immunoassay and rapid cell culture are efficient and sufficiently sensitive for the diagnosis of adenovirus enteritis and upper respiratory disease in immunocompetent children [43]. In immunocompromised patients, however, PCR-based assays have been established as a standard diagnostic tool for rapid, specific, quantitative, and highly sensitive detection of HAdV in any diagnostic material [42, 44–49]. The analysis of peripheral blood samples of immunocompetent children with HAdV-associated respiratory disease and of healthy controls showed that HAdV DNA may be detected in about 40% of children with respiratory HAdV infection for a median period of 4.5 days and occasionally in healthy individuals at a very low level [50].

In neonates and immunocompromised patients, HAdV can cause lethal organ damage [51]. One of the first lethal HAdV infections published was the case of a child with severe congenital immunodeficiency [52]. HAdV infections in patients receiving chemotherapy seems to be a rare event, but lethal disseminated HADV disease has been described in this condition [53–59].

In adult HIV patients, acute diarrhea is frequently associated with HAdV positivity in stool, whereas in HIV positive children HAdV does not seem to be an important cause of diarrhea [60, 61]. There are only sporadic reports on severe or lethal HAdV infections in HIV patients [62, 63].

33.2 Diagnostics

During the last years real-time PCR assays for detection and quantification of HAdV has become the gold standard in the context of allogeneic HSCT [5, 42, 44, 46, 47, 64, 65]. The difficulty of targeting the wide range of serotypes with major genetic differences between the species has been overcome by designing primer/probe combinations derived from the hexon and the VA RNA region [45, 47, 49]. There are a number of commercial kits available. In-house quantitative PCR seem to be comparable to those achieved with commercial kits [49, 66, 67]. The most commonly identified species in the context of HSCT are C (serotypes 1, 2, 5, 6), followed by A (serotype 31) and B (serotypes 3, 11, 34), although in rare instances D and F can be detected [68–71]. Sequential or simultaneous infections with different species have been described occasionally [1, 68–71].

As it has been shown that detection of high levels of HAdV DNA in peripheral blood predicts disseminated HAdV disease, a quantitative HAdV PCR-screening in peripheral blood samples has become standard in many centers [44, 46, 47, 70, 72–74].

The screening for HAdV in the stool and molecular monitoring of viral load in serial stool specimens facilitate early detection of impending invasive infection [1, 66, 74, 75]. It is important to bear in mind, however, that inhibitors of PDR amplification may be present in stool specimens and the implementation of appropriate controls is necessary to prevent false negative results. In the guidelines published in 2012 by the European Conference of Infections in Leukemia (ECIL) monitoring with quantitative PCR of HAdV load in peripheral blood on a weekly basis for all patients with at least 1 risk factor is recommended and the duration of monitoring should be adapted to degree of immune reconstitution [76].

33.3 Incidence

Shields et al. were the first to describe the impact of HAdV on allogeneic hematopoietic stem cell transplantation (HSCT) [53]. During the last decade, HAdV infection in the context of allogeneic stem cell transplantation has been increasingly recognized as a major cause of transplant-related mortality (TRM) especially in children undergoing allogeneic HSCT. Whether this "increase" is due to a higher percentage of T-cell–depleted grafts and highly efficient peritransplant immunosuppression or due to the fact that with the use of highly sensitive PCR techniques HAdV is increasingly being diagnosed remains open.

The first study based on HAdV-PCR screening in stool, urine, and throat swab samples was performed by Chakrabarti et al. in a cohort of 76 adult HSCT patients [77]. A number of screening studies have been performed since then, mainly in pediatric HSCT patients, revealing an overall incidence of HAdV infection between 12% and 42%. Although 55–60% of the infected patients remained asymptomatic, an incidence of lethal disease between 0.9% and 7% has been observed [1, 47, 70, 72, 73, 77–84]. The broad range of reported incidences may be due to the differences concerning the diagnostic samples (peripheral blood, feces, urine) and the diagnostic approach (culture, PCR). The reported screening studies based on PCR screening in peripheral blood since the year 2000 are summarized in Table 33-1.

TABLE 33-1. Screening studies on incidences of HAdV viremia and HAdV-related mortality [1, 47, 70, 72, 78, 82–84, 86, 94, 95, 109]

Author	Year	Pts	Children	HAdV viremia (%)	Lethal disease (%)
Lion et al.	2003	132	132	8	72
Leruez-Ville et al.	2004	58	58	26	0
Walls et al.	2005	26	26	42	18
Kampman et al.	2005	155	155	17	19
Takayama et al.	2007	32	16	33	17
Myers et al.	2007	22	20	68	n.r.
Gustafson et al.	2008	40	13	15	50
Bin-Lula et al.	2010	116	84	26	17
Lion et al.	2010	153	153	10	50
Öhrmalm et al.	2011	97	20	5	0
Hiwarkar et al.	2011	291	291	15	n.r.
Sive et al.	2012	116	0	12	7

33.4 Risk Factors

Several risk factors for HAdV infection and, more importantly, HAdV disease have been identified.

33.4.1 Patient Characteristics

Although HAdV infections are more frequently reported in children, recent screening studies revealed incidences of viremia between 5% and 12% [70, 72, 84–86]. Within the pediatric HSCT patient cohort, however, younger age seems to be a significant risk factor [75, 81]: Although similar rates of HAdV infections are observed in adult HSCT patients, the clinical impact of HAdV infections in adult HSCT patients remains controversial [72, 84, 87–90]. Recipient and/or donor serostatus do not have an impact on the occurrence of HAdV infection [91, 92]. However, HAdV PCR positivity in nasopharyngeal aspirate preceding hematopoietic stem cell transplantation or HAdV infection prior to SCT seem to be a very strong risk factor for adenovirus DNAemia in pediatric patients [93, 94].

33.4.2 Donor and Graft

Patients transplanted with unrelated cord blood, and patients transplanted from unrelated donors (who usually receive in vivo or ex vivo T-cell–depleted grafts and have a higher incidence of severe GvHD) have an increased risk for HAdV infections and disease [83, 95–98]. This underlines the impact of prolonged immunosuppression on posttransplant HAdV infection, attributable either to in vivo or ex vivo T-cell depletion, prolonged lymphopenia, or to GvHD-associated immunosuppressive posttransplant therapy [78, 82, 99]. In a study published by van Tol et al. the risk of HAdV infection, disease, and lethal outcome increased with the degree of T-cell depletion from 8%, 2%, and 0% (<2 log T-cell depletion) to 29%, 18%, and 7% (>2 log T-cell depletion) and 71%, 42%, and 42% (>3 log T-cell depletion), respectively [81]. Lee et al. found in a restrospective case finding study in 624 adult and pediatric patients a tenfold increased incidence of HAdV disease in patients receiving T-cell depleted grafts [79]. Analyzing patients with or without ATG-containing conditioning regimens (irrespective of the graft manipulation), Runde et al. reported a significantly higher incidence of HAdV infection in patients receiving ATG [92]. Several groups have addressed the question of whether the mode of T-cell depletion (i.e., ex vivo, in vivo, specific antibodies) has an impact on the risk of HAdV infection: Lion et al. compared patients who received pretransplant ATG and an unmanipulated graft with those who received ATG and an ex vivo T-cell–depleted graft and found a significantly higher incidence of HAdV infection in patients with ex-vivo T-cell depleted grafts; 8 of 11 patients who experienced lethal disease had received ex vivo T-cell–depleted grafts [47]. Myers et al. compared the incidence of HAdV infection and HAdV disease in pediatric patients with either ATG- or alemtuzumab containing conditioning regimens: patients receiving alemtuzumab had a significantly higher probability at 6 months of HAdV infection (35% vs. 13%) and HAdV disease (14% vs. 2%) compared to patients receiving ATG [80], although the impact of serotherapy on the incidence of HAdV infections may be influenced by the dose and length of exposure [100]. The impact of alemtuzumab containing conditioning regimens on HAdV disease in adult patients is being discussed controversially: Sive reported a HAdV associated mortality of only 7%, whereas Avivi and others found a high HAdV-related mortality of 50%, despite a comparable infection rate [77, 84, 85, 88, 89].

33.4.3 Graft-Versus-Host Disease

Severe acute graft-versus-host disease (GvHD) requiring intensified or prolonged immunosuppressive treatment seems to be associated with an increased risk for HAdV infection and disease [1, 60, 79, 80, 85, 92, 96]. The lack of correlation of HAdV infection with GvHD reported in two large screening studies may be due to the fact that these included a large proportion of patients who had received a T-cell–depleted graft [47, 77]. Patients undergoing T-cell–depleted HSCT usually do not experience GvHD but have an increased risk of HAdV infection, which may result in a statistically similar incidence of HAdV infection and disease in patients with or without GvHD. Excluding patients with T-cell–depleted grafts from the statistical analysis reveals a significant correlation between severe GvHD and HAdV infection and disease.

33.4.4 Delayed Immune Reconstitution

There is an obvious correlation of delayed T-cell reconstitution and the risk of HAdV infection [77]. The association of HAdV infection with low T-cell counts has been confirmed in several studies [81, 83, 94, 101]. van Tol et al. showed in a homogenous group of patients with comparable immunosuppressive therapy and comparable risk for GvHD that individuals with delayed T-cell recovery have a significantly higher risk of HAdV infection and, more importantly, progression to disease [81] The monitoring of qualitative (detection of HAdV-specific T-cells) instead of quantitative (total T-cell count) seems to be a more elegant approach to evaluating the risk for progression from HAdV infection to HAdV disease [81, 95, 101, 102]. The detection of HAdV-specific T cells either by IFN-γ ELIspot assays, CD40L upregulation or multimer staining may help clinicians to identify those patients who are shedding HAdV or display HAdV viremia but are at low risk for HAdV disease [102–105].

33.5 Transmission and Reactivation

Viral infections in the context of allogeneic stem cell transplantation are supposed to be the result of transmission by the stem cell graft, endogenous reactivation, nosocomial, or community-acquired transmissions.

It has been suggested, that HAdV infections may be donor derived because donors of HSCT patients developing HAdV infection were seropositive and HAdV could be detected in the graft in rare occasions. However, this could not be confirmed in other studies [92, 95, 106]. The fact that different HAdV serotypes are detectable simultaneously or sequentially in a number of HAdV-infected patients does not support the hypothesis of transmission via the stem cell graft [1, 68, 71, 107].

Although HAdV disease usually occurs between 2 and 3 months posttransplant, screening studies in the pediatric setting showed that the median time point of the first HADV detection in peripheral blood is day +15 [1, 108]. As HAdV is usually detected for the first time when patients are still nursed under strict protective isolation measures, and no seasonal distribution can be observed, community-acquired infections are unlikely in most patients.

However, several HAdV outbreaks in HSCT units have been described, some with and some without evidence of nosocomial origin [109–112].

Several findings support the hypothesis that HAdV infection post transplant is caused by endogenous viral reactivation in most cases. In contrast to immunocompetent patients, adenovirus infections in immunocompromised patients present no seasonal variation [37]; high titers of HAdV antibodies against a certain serotype in the recipient prior to SCT have been shown to be associated with HAdV infections with the same serotype after SCT [113]; infectious HAdV strains detected in children before transplant are identical with the strain isolated after HSCT [1, 39, 81, 114]. This is supported by the observation that adenoviral DNA is highly prevalent in lymphocytes from the gastrointestinal tract and HAdV viremia is preceded by viral shedding in stool in most cases [1, 41, 46, 115, 116]. Once infection has occurred, viral load can increase rapidly with a viral doubling time of 1–3 days [117]. In an adult transplant patient, it has been shown that subsequent virus replication in the colon, bone marrow, and liver was the origin of HAdV DNAemia [118].

33.6 Definitions of HAdV Disease

As HSCT patients, especially those who are severely immunosuppressed due to T-cell depletion or GvHD, frequently have several possible causes for their symptomatology, it may be difficult to attribute specific symptoms or even lethal organ failure exclusively to HAdV in the context of GvHD or other viral and fungal infections. With the perception that HAdV is an important pathogen in the HSCT setting, several attempts have been made to clearly define HAdV infection and disease [115, 119, 120]. It is an established consensus that infection is defined as the detection of HAdV by any method at any site. Definite disease has classically been defined as clinical symptoms compatible with HAdV infection together with either detection of HAdV in tissue culture or histologic evidence; probable disease was defined by most authors as appropriate symptoms, together with either the presence of HAdV in the absence of other recognizable causes or with the HAdV isolation from tissue site with or without histologic evidence. Disseminated disease has been defined by some authors as HAdV viremia, by others as multiple organ involvement in the presence of two or more HAdV-positive PCR assays in PB and other sites tested, in the absence of other identifiable causes [1, 81]. HAdV-related death is usually defined as the detection of HADV in biopsy specimens at autopsy [77, 119].

Taking into account that highly sensitive PCR techniques have become the diagnostic gold standard for HAdV in the context of allogeneic HSCT, ECIL the following definitions have been recommended by ECIL [76]:

- Systemic infection/viremia: Positive HAdV PCR, virus isolation, or antigen detection in peripheral blood.
- Local infection: Positive HAdV PCR, virus isolation, or antigen detection in biopsy material or in body fluids other than peripheral blood.
- Probable disease: HAdV infection plus corresponding symptoms and signs without histological confirmation.
- Proven disease: HAdV infection plus corresponding symptoms related to the infection and histological confirmation of HAdV in the appropriate location.

33.7 Clinical Manifestation

To date, the pathogenetic mechanism of HAdV disease is not completely understood. It has been shown in several animal models that innate cellular immune responses and the respective proinflammatory cytokines largely contribute to the HAdV-associated organ damage, especially in the liver [121, 122]. The binding of HAdV to circulating platelets, which causes their activation/aggregation and subsequent entrapment in liver sinusoids may also contribute to hepatic symptoms and the frequently observed hemorrhagic events [123]. Additionally, HADV seems to be able to induce auto- or alloimmune mechanisms [124].

The first symptoms of HAdV disease in HSCT patients frequently are diarrhea, fever, elevated liver enzymes, and secondary pancytopenia.

However, it has been shown in screening studies that the detection of HAdV in stool samples is associated with enteric symptoms in only 20–30%[1, 81].

The spectrum of HAdV-associated disease in HSCT patients ranges from mild enteritic or respiratory symptoms to severe hemorrhagic enteritis, hemorrhagic cystitis, nephritis, hepatitis, pneumonia, encephalitis, myocarditis, pancreatitis, and lethal multiple organ involvement, which frequently is associated with hepatic failure [31, 73, 85, 87, 89, 90, 125–128]. Although not all patients who are HAdV PCR positive in peripheral blood become symptomatic, all reported lethal cases developed viremia and it has been shown in several studies, that increasing or high viral load is associated with lethal HAdV disease [1, 70, 72, 79, 83, 84, 90]. There is frequently an asymptomatic interval of several weeks between the detection of HAdV viremia and the progression to invasive disease. The occurrence of HAdV viremia prior to day + 50 seems to be associated with a poor outcome [74]. Finally there is a significant correlation between HAdV infection and disease and overall TRM [129]. In a mouse model, it has been shown that in the presence of HAdV, lipopolysaccharide-induced TNF production rises dramatically in peripheral blood, liver, spleen, and lung, and lethality increases after lipopolysaccharide challenge. This observation suggests that HAdV infection might increase the septic mortality and thus indirectly contribute to TRM [130, 131].

33.8 Prevention and Therapy

33.8.1 Prevention

Strict isolation and hygiene measures in HSCT patients shedding the virus are absolutely necessary in order to prevent horizontal transmission and nosocomial outbreaks. Sodium hypochlorite for 10 min or 85% ethanol for at least 2 min can inactivate HAdV [132, 133]. The CDC guidelines recommend contact precautions such as private room, masking, gowning, and gloving in order to minimize the risk of transmissions to other patients and contamination of surfaces [134].

No randomized trials on the use of immunoglobulins as prophylaxis for HAdV infections are available. It has been shown however, that immunoglobuline prophylaxis does not have any impact on the incidence of post-transplant CMV infection [135].

In some pediatric HSCT centers, patients with positive HAdV-PCR in stool prior to T-cell–depleted HSCT receive one dose of cidofovir prior to conditioning, thus taking the opportunity of treatment prior to deletion of the host immune system (R. Handgretinger, personal communication).

33.8.2 Pharmocological Therapy

For three commercially available antiviral agents—ganciclovir, ribavirin, and cidofovir—in vitro efficacy against different HAdV serotypes has been shown [136, 137].

Ganciclovir displays some in vitro activity against HAdV, and one study showed a correlation between HADV infection and the absence of prophylactic or preemptive ganciclovir treatment [138].

Ribavirin is a purine nucleoside analogue with in vitro activity against most HAdV isolates from species A, B, and D, and in all isolates from species C [136]. Although viral clearance has been described in a retrospective analysis of immunocompromised children with severe HAdV disease, no therapeutic benefit could be observed in a cohort of pediatric patients with HAdV viremia [139, 140].

Cidofovir, a cytosine analogue that inhibits DNA polymerase activity with comparable in vitro activity against different HAdV subgroups, has become standard treatment for HAdV infections [141, 142]. It is used as induction therapy in a dose of 5 mg/kg/week and 5 mg/kg every 2 weeks thereafter. Cidofovir-associated nephrotoxicity, cytopenia, and uveitis may contribute substantially to the HAdV-associated morbidity [119]. Hyper-hydration and the concomitant administration of probenecid is mandatory in order to reduce nephrotoxicity. Patients treated with cidofovir must undergo frequent monitoring of renal and especially tubular function. Dose reduction (1 mg/kg three times weekly) seems to be associated with less nephrotoxicity, but the effectiveness remains open [91, 143].

Cidofovir seems to be associated with viral clearance and clinical recovery in non-immunocompromised patients with HAdV pneumonia [144].

Although successful treatment of HAdV infection and disease following HSCT with cidofovir has been reported in several retrospective and prospective studies, the therapeutic value in the context of HSCT and—more importantly—severe lymphopenia remains open. No randomized trials are available and published data are based on extremely heterogenous patient populations concerning T-cell depletion and the presence of severe GvHD, different time-points of infection (ranging from pretransplant infection and very early disease up to more than 200 days posttransplant), different treatment regimens, and a variety of concomitant measures such as withdrawal of immunosuppression or DLI. Retrospective analyses revealed that up to 40% of HSCT patients receiving cidofovir for viral infections other than HAdV develop HAdV viremia during cidofovir treatment [74, 119]. The antiviral effect of cidofovir treatment seems to be strongly influenced by T cell reconstitution [145, 146].

The outcome of patients treated for HAdV viremia with cidofovir is summarized in Table 33-2.

The compound brincidofovir (CMX001; 1-O-hexadecyloxypropyl-cidofovir) is an orally bioavailable lipid conjugate of cidofovir, was able to eradicate disseminated HAdV infection in a mouse model and its safety has been demonstrated in healthy volunteers [147, 148]. However, data on the clinical effectiveness in the context of HAdV is still very limited [149, 150].

Table 33-2. Outcome of cidofovir treatment for patients with HAdV viremia: HAdV-related mortality [1, 47, 74, 84, 87, 88, 96, 108, 109, 116, 145, 161–166]

Author	Year	No. of Pts	HAdV-related mortality (%)
Legrand et al.	2001	1	0
Lion et al.	2003	8	100
Lereuz-Ville et al.	2004	6	0
Muller et al.	2005	6	16
Yusuf et al.	2006	57	18
Kalpoe et al.	2006	7	43
Symeonidis et al.	2007	3	67
Robin et al.	2007	25	68
Neofytos et al.	2007	6	33
Omar et al.	2010	13	15
Lion et al.	2010	16	50
Verdeguer et al.	2011	28	11
Taniguchi et al.	2012	5	20
Watson et al.	2012	6	33
Sive et al.	2012	5	20
Mynarek et al.	2014	45	4
Lugthart et al.	2015	36	8

The results of a prospective double blind placebo controlled phase II trial on preemptive treatment for HAdV viremia (The HAdv Halt Trial, https://clinicaltrials.gov/ct2/show/NCT01241344) have not been published to date.

A prospective study on the preemptive treatment with CMX001 in children with high viral load in stool samples is currently under way.

33.8.3 Adoptive Transfer of HAdV-Specific T Cells

Feuchtinger et al. showed that patients who cleared HAdV infection displayed HAdV-specific T cells until day 200 post-HSCT at significantly higher frequencies than patients who failed to control HADV infection [104]. Heemskerk et al. found that the survival of patients with HAdV viremia was associated with an increase in lymphocyte counts together with the presence of HADV-specific $CD4^+$ T-cell responses and increases in titers of neutralizing antibody [101]. The fatal outcome of HAdV disease despite pharmacological treatment, together with the findings that outcome is mainly related to HAdV-specific immunoreconstitution, suggests that the recovery of HAdV-specific immunity is critical for successful antiviral therapy.

These observations and the fact that the transfer of virus specific T cells is feasible and successful in other posttransplant viral infections have prompted several attempts to select donor-derived HAdV-specific T cells [151–153].

HAdV-specific T-cells are supposed to be cross-reactive with all HAdV subtypes, because the hexon is the immunodominant T-cell target among HAdV capsid proteins, and contains multiple epitopes conserved among different serotypes [19, 24]. In vitro experiments showed that HAdV-specific T cell can be generated by HAdV-pulsed dendritic cells and that cytotoxic T cells raised against HAdV species C can lyse cells infected with species B [18, 151, 154]. Feuchtinger et al. isolated HAdV-specific T cells through an INF-γ secretion and capture assay and could show specific antigen responses of both $CD4^+$ and, to a lesser extent, $CD8^+$ T cells by INF-γ expression and cytotoxicity assays upon restimulation with different HAdV strains [153].

In general, the selection of HAdV-specific T-cells is based either on long-term expansion [107, 155], magnetic separation of virus-specific T-cells using the IFN-γ-capture assay [156] or multimers [103].

Other promising approaches are the allodepletion of donor T cells to improve immune reconstitution concerning all common viruses, the generation of third party T-cells specific for the three most prevalent viruses in the context of HSCT by transduction with a clinical grade HAdV vector and restimulation with irradiated EBV-transformed lymphoblastoid cell lines or the transfection of γ/δ donor-T-cells with adeno-specific T-cell receptor by electroporation [157–159].

In a pilot-study Feuchtinger et al. treated six patients with HAdV viremia with virus-specific donor T cells generated by INF-γ secretion assays [156]. In three of four evaluable patients the infused T cells underwent an in vivo expansion and the viral load decreased in peripheral blood after adoptive T-cell transfer. In vivo expansion of specific T cells was dose independent, suggesting that even very low numbers of HAdV-specific donor T cells expand easily in vivo in the presence of viremia.

Although only few clinical phase I/II trials have been published so far, they showed impressive clinical results concerning reduction of viral disease without inducing severe GvHD. Clinical trials and trial results are summarized in Table 33-3.

33.9 Preemptive Treatment Strategies

In the view of the fact that HAdV viremia is frequently followed by lethal disease, the availability of highly sensitive screening methods and a “window of opportunity” between detection of HAdV and HAdV disease, early preemptive treatment in patients at risk is strongly recommended. However, considering the substantial toxicity of cidofovir and the high percentage of patients shedding HAdV in stool without becoming symptomatic, preemptive treatment for all patients with PCR-positive stool or blood samples is not recommended. Taking into account the high risk of HAdV viremia in patients with high viral loads in stool, preemptive treatment strategies based on quantitative PCR in stool samples may be considered [1, 75]. As severe lymphopenia and delayed immunoreconstitution are risk factors for HAdV disease, all guidelines and algorithms strongly recommend

TABLE 33-3. Adoptive therapy with HAdV-specific T-cells [107, 155, 156, 159, 167–171]

	Method	Donors/infused cell number	Outcome
Feuchtinger et al. (2006)	Interferon-γ capture	Stem cell donors (unrelated) 1–50/10E3/kg	GvHD 1/9, HAdV 1/6 resolved
Leen et al. (2009)	Culture with transduced EBV-transformed B lymphoblastoid cell lines	Stem cell donors (unrelated and haplo) 10E6–10E8/m^2	GvHD 0/12, HAdV 2/2 resolved
Uhlin et al. (2012)	Selection with HLA multimers	Third party 3×10E4/kg	GvHD nr, HAdV 0/1 resolved
Gerdemann et al. (2013)	Short-term plasmid-stimulated culture (CMV, ADV, EBV)	Stem cell donors (unrelated and haplo) 0.5–2×10E7/m^2	GvHD 0/10, HAdV 5/5 resolved
Quasim et al. (2013)	Interferon-γ capture	Stem cell donors (unrelated and haplo), third party 1×10E4/kg	GvHD 1/5, HAdV 3/5 resolved
Leen et al. (2013)	Culture with transduced EBV-transformed B lymphoblastoid cell lines	Third party 2×10E7/m^2	GvHD 8/45, HAdV 14/17 resolved
Geyeregger et al. (2014)	Short-term culture of HAdV stimulated donor T-cells	Stem cell donors (haplo) 1×10E4/kg	GvHD 1/2, HAdV 1/2 resolved
Di Nardo et al. (2014)	Interferon-γ capture	Stem cell donors (haplo) 1×10E5/kg	GvHD 0/1, HAdV 1/1 resolved
Feucht et al. (2015)	Interferon-γ capture	Stem cell donors (sibling, unrelated, haplo) 4×10E3/kg	GvHD 0/30, HAdV 22/30 resolved

TABLE 33-4. Screening and preemptive treatment strategies [1, 76, 120, 142]

	Patients to be screened	Diagnostics	Preemptive treatment with cidofovir	Immunotherapy
Matthes-Martin et al. (2012)	Allo HSCT with ≥1 risk factor	Weekly PB	If ≥1 risk factor	Reduce IS if possible
Lion et al. (2010)	All pediatric allo-HSCT	Weekly PCR screening in feces	All: viral load >10E6/g feaces	Reduce IS if possible
	If viral load >10E6/g faeces	Weekly PCR screening in PB	Increasing viral load despite treatment	HAdV CTLs
Lindemans et al. (2010)	All pediatric allo-HSCT	Weekly PCR screening in PB	High-risk patients: viral load >10E2	Reduce IS if possible
	PCR positive patients	Twice weekly	Intemediate-risk patients: viral load >10E3	Reduce IS if possible
			Low-risk lymphopenic patients: >10E3	Reduce IS if possible
			Low-risk patients: >10E4	Reduce IS if possible
			Increasing viral load despite treatment	HAdV CTLs
Chakrabarti et al. (2004)	All allo-HSCT	Weekly: PB	Any	Reduce IS if possible
		Weekly: feces, urine, throat	If symptomatic: any	Reduce IS if possible
			If asymptomatic and lymphopenic: any	Reduce IS if possible
			If asymptomatic and immuosuppressed: any	Reduce IS if possible
			If no improvement	HAdV CTLs

the reduction of immunosuppressive therapy, whenever possible [76, 120, 142, 160]. All proposed treatment strategies today, recommend third-line therapy with virus-specific T-cells. This should, however, be performed exclusively in the context of prospective trials and restricted to experienced centers until it is confirmed that the transfer of HAdV-specific T-cells is feasible, safe, and effective.

Several algorithms for preemptive treatment in case of DNAemia have been proposed with the aim to prevent HAdV-associated mortality on one hand and overtreatment on the other hand. Published preemptive treatment strategies are listed in Table 33-4.

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34
Adenovirus Infection in Solid Organ Transplantation

Marian G. Michaels, Michael Ison, and Michael Green

34.1 Epidemiology

Adenoviruses are nonenveloped, double-stranded DNA viruses [1]. There are 57 immunologically distinct types of adenoviruses that are further classified into one of seven (A–G) subgroups based on hemagglutinin properties, DNA homology, oncogenic potential in rodents, and clinical disease (Table 34-1) [2, 3]. Infections due to adenovirus occur throughout the year without significant seasonal variation. The highest incidence of adenoviral infection occurs in children between the ages of 6 months and 5 years [2, 4, 5], although outbreaks have been noted historically among military recruits, adolescents at summer camp, and sometimes nursing home residents [6, 7]. Explanations for the infrequent incidence of disease in the very young or in older individuals include the presence of transplancentally acquired maternal antibody in the young infant and the development of neutralizing antibody to the most common adenoviral strains (serotypes 1, 2, and 5) in the majority of children older than 5 years [2]. A risk factor for infection in the preschool age group is the increased likelihood of person-to-person spread resulting from intimate contact between children in the absence of well-developed sanitary habits, especially those in day care or other closed environments [4]. Similarly, it is thought that the relative lack of hygiene and crowding in military barracks is the cause of the increased risk of adenoviral spread among recruits.

Adenovirus has been reported in recipients of all organ types and recipients of bone marrow transplantation with wide ranges depending on the organ transplanted, the age of the patient and the type of study [2–27]. In general infection has been seen more commonly in children compared to adult organ recipients [8, 23, 28]. Presumably, this difference is due to adults having preexisting immunity against adenovirus. However, serological evidence of previous infection does not confer complete protection against invasive adenovirus disease (e.g., adenovirus hepatitis has occurred in pediatric LT recipients with type-specific antibody) [10]. In addition, it is sometimes difficult to attribute specific disease states to the finding of adenovirus since it can be shed asymptomatically for long periods of time even in the normal host [2] Descriptions of adenoviral infections associated with specific types of SOT are provided later in this chapter.

34.2 Mode of Transmission

Adenovirus is typically transmitted person-to-person via direct contact, airborne droplets, or fomites. Among transplant recipients, disease is most frequently described as occurring within the first few months after transplantation. Although outbreaks of adenovirus infections among transplant recipients have been reported [12], attempts to document nosocomial person-to-person spread in two large series were unsuccessful [8, 13]. Adenovirus has not been reported to spread through the use of blood products, thereby eliminating another potential source of viral transmission, though the possibility of transmission via stem cell products has been raised. These facts, as well as the stereotypical timing of adenoviral infection within the first several months after transplantation [8, 13, 14], have led to the suggestion that reactivation of latent virus and/or donor organ-associated transmission are the major sources of early infection in transplant recipients. In support of this hypothesis is the capacity of adenovirus to remain latent and the striking similarities, in terms of both timing of infection and relationships between serostatus and severity of disease [10], that exist between adenovirus and cytomegalovirus. Of greatest similarity is the significant increase in organ-specific adenoviral disease in the transplanted organ. However, the early diagnosis of adenovirus may in part also be biased by more frequent testing early after transplantation.

34.3 Clinical Disease

A number of clinical reports of adenovirus infection after SOT have been published [8–11, 14–27, 29–34]; a summary of their findings is shown in Table 34-1. While manifestations can vary by organ type, several general comments

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Table 34-1. Adenovirus serotypes associated with specific symptoms in immunocompromised hosts

Host	Disease state	Common serotype
Immunocompromised hosts	Persistence in urinary tract Persistence in colon Pneumonitis, hepatitis	4, 11, 34, 35 42–49
AIDS	Gastroenteritis, pneumonitis, hepatitis, encephalitis	1, 2, 5, 11, 26, 28, 29, 30, 37, 43–47
Bone marrow/stem cell transplant	Gastroenteritis, pneumonitis, hepatitis, hemorrhagic cystitis, encephalitis, disseminated disease	1–3, 5, 7, 11, 31
Liver transplant	Gastroenteritis, hepatitis, pneumonitis, disseminated disease	1, 2, 5, 7, 31
Renal transplant	Hemorrhagic cystitis, pneumonitis, gastrointestinal disease	11, 34, 35
Lung transplant	Pneumonitis, disseminated disease	2, 5[a]

[a]Serotyping often not reported in the literature.

Table 34-2. Definitions of adenovirus infection and disease

Adenovirus disease status	Definition
Asymptomatic infection	Detection from stool, urine, respiratory secretions or blood by culture, antigen test, or PCR in the absence of signs or symptoms of illness
Possible adenovirus infection	Detection in above sites or in the cerebrospinal fluid or bronchoalveolar lavage in the presence of signs or symptoms of illness but with concurrent infection or rejection of the affected organ
Probable adenovirus infection	Same as possible but without concurrent infection or rejection of the affected organ
Proven adenovirus invasive infection	Biopsy proven with histologic evidence of adenovirus in tissue
Disseminated adenovirus Infection	More than two organ systems infected not including blood

Modified from Florescu DF, Hoffman JA and the AST Infectious Diseases Community of Practice, Adenovirus in solid organ transplantation, Am J Transplant. 2013;13(Suppl 4):206–11. doi:10.1111/ajt.12112.

pertain to all organs. First, as mentioned previously, confirming that observed clinical symptoms are due to adenovirus can be difficult as this virus can shed asymptomatically or be carried latently. Definitions categorizing clinical manifestations of adenovirus after transplantation have been proposed by the American Society of Transplantation Infectious Diseases community of Practice [28]. These include asymptomatic shedding, symptomatic disease, or disseminated disease; Table 34-2 expands this to include possible, probable, and proven disease [28].

34.3.1 Liver Transplantation

Historically, adenovirus had been recognized as the third most important viral pathogen (after cytomegalovirus (CMV) and Epstein–Barr virus (EBV)) in children undergoing liver transplantation, accounting for infection in 10% of 484 pediatric LT recipients under cyclosporine A in our center in the 1980s [8]. We subsequently observed a dramatic decrease in the incidence and severity of adenovirus infection after the introduction of tacrolimus-based immunosuppression. A review of more recent experience in the literature appears to support this decline in the importance of adenovirus in this population. Key epidemiologic observations from the earlier series of adenovirus in pediatric liver transplant recipients under cyclosporine-based immune suppression included the development of disease in younger recipients (median age of l 3.0 years; range 0.8–17.2 years) and the development of infection early after transplant [8–11]; the median time of adenoviral isolation was 25.5 days after LT in our series, with severe infections typically presenting in the first 50 days [8]. Symptomatic disease, ranging from self-limited fever, gastroenteritis, or cystitis to devastating illness with hepatitis or pneumonia, was observed in 60% of pediatric LT recipients with evidence of adenoviral infection [8]. Hepatitis was the most common form of invasive infection, and was associated with prolonged high-grade fevers. Eight of 14 pediatric LT recipients with adenoviral hepatitis recovered including four who lost their graft during the course of the disease and underwent successful retransplantation. One of the four had a mild episode of recurrent disease. Adenovirus pneumonia was also seen in this series affecting 8 of 48 pediatric LT recipients; 6 of these 8 children died, including 2 with concurrent hepatitis. Serotypes 1, 2, and 5 were the most common isolates in this series [8] with serotype 5 being the primary cause of hepatitis and serotype 2 associated with the most cases of pneumonia. The use of OKT3 was identified as a risk factor for symptomatic disease [8]. The presence of donor/recipient serologic mismatching was associated with development of both infection and more severe disease [9, 10].

A single report describes the experience with adenovirus infection in adult LT recipients [9]. Eleven of 191 (5.8%) adult recipients of LT had positive cultures for adenovirus in this series. However, only 7 of the 11 were thought to have developed symptomatic disease. In three of the seven symptomatic cases, disease was limited to the urinary tract. Disseminated disease associated with pneumonia was observed in three patients. The remaining patients developed fulminant hepatitis. Death was attributable to adenovirus in one case of disseminated disease with pneumonia and in the case of fulminant hepatitis. The mean time to isolation of adenovirus by culture was 66 days after transplant, while the mean time to onset of symptomatic disease due to adenovirus was 55 days (range 3–180 days) after transplant. A second report describing results of surveillance for adenovirus in adult SOT recipients

found that 8.3% of 121 liver recipients had AV detectable by PCR in their blood in the first 12 months after LTx. However, the majority were asymptomatic, and progression to significant disease did not occur [10].

34.3.2 Renal Transplantation

The reported experience of adenovirus infections after RT is limited to case reports that have accumulated over the last 25 years [27] The most frequently reported clinical syndrome has been hemorrhagic cystitis due to adenovirus types 7, 11, 34, and 36 [14, 19–22, 27]. Generally, episodes are self-limited despite the fact that many of these patients were initially treated as having rejection with augmented immunosuppression. Hemorrhagic cystitis sometimes accompanied with adenovirus nephritis may be confused with renal rejection. Patients with adenovirus nephritis generally present with persistent fevers, microscopic or macroscopic hematuria, and elevated serum creatinine [35]. More serious illness, consisting of interstitial pneumonia has been less frequently reported but is associated with a higher rate of fatal, disseminated disease [16–18]. Additionally, a single case of fatal hepatitis due to adenovirus type 5 has been reported following RT [15].

Similar to adenoviral infections after pediatric LT, the vast majority of adenovirus infections after RT have developed within the first 6 months after transplantation (range 17 days to 9 months; median 2.5 months) [14–22, 27]. However, all the reported cases of adenoviral infection in RT recipients have occurred in adults (range 17–61 years; median 30.5 years) [14–22]. The early timing after transplantation and the predilection for infection of the transplanted organ has again led to the hypothesis that these cases may be due to donor associated transmission. This may represent publication bias, though, as clinical experience suggests milder, self-limited infections can occur throughout the entire post-transplant period.

34.3.3 Cardiac Transplantation

There is less information about adenoviral infection following cardiac transplantation compared to other organ types [26]. However, adenovirus genome has been identified by polymerase chain reaction in endomyocardial biopsy specimens of 8 of 40 pediatric cardiac transplant recipients who had histologic evidence of inflammation indistinguishable from rejection [36]. Of interest, several of the children with this finding had recent or concurrent histories of upper respiratory tract infections. Two of the eight patients presented with cardiogenic shock and were felt to have histologic evidence of high-grade rejection. They were treated for rejection with improved clinical status, and adenovirus could not be identified on subsequent biopsies. Whether adenovirus was the sole cause of the inflammation, a promoter of rejection, or an innocent bystander in these patients was not established in this report. An additional report among pediatric heart transplant recipients identified an association between detection of adenoviral genome copies in myocardial biopsy specimens and adverse clinical events including coronary vasculopathy and graft loss [37]. As with the previous report, causal relationships between the presence of adenoviral DNA and the subsequent development of these adverse events remain to be proven. Anecdotal case reports of adenovirus disease after heart transplantation have been published in both adults and pediatrics [30, 31].

34.3.4 Lung Transplantation

Adenovirus disease after lung transplantation appears to have higher morbidity and mortality than seen with adenovirus and other organ types. Ohori et al. reported the outcome of four cases of adenovirus pneumonia among 308 lung transplant recipients at the University of Pittsburgh [23]. As noted with other organ transplant recipients, the incidence was much higher among children (3 of 40) than among adults (1 of 268) [23]. Disease occurred in the first 6 weeks following transplantation and was uniformly fatal. Dissemination beyond the respiratory tree was not demonstrated despite the performance of an autopsy in each of the patients. The authors speculated that the presence of ischemic harvest injury predisposed their patients to the development of adenovirus pneumonitis.

Bridges et al. reported their experience with adenovirus infection in pediatric lung and heart-lung transplant recipients [32]. Adenovirus was identified in the lung of 8 of 16 patients occurring between 1 and 458 days following transplantation. Adenovirus was associated with early, fulminant infection in two patients. Perhaps as importantly, these and other investigators found that adenovirus infection of the transplanted lung was significantly associated with respiratory failure leading to graft loss or death and with the histologic diagnosis of obliterative bronchiolitis [32, 38].

34.3.5 Intestinal Transplantation

Several reports have documented a very high rate of adenoviral infection among pediatric intestinal transplant recipients with rates ranging from 20.8% to 100% [24, 25, 33]. In contrast, only limited experience with adenovirus infection has been reported in adult intestinal transplant recipients [34]. At least one of the reports of high rates of adenoviral infection may be confounded by the fact that viral cultures were obtained as part of routine screening of graft biopsies and not all of infected patients were symptomatic. The interpretation of a positive adenovirus culture or histologic finding is made difficult by the frequent absence of any associated symptoms

in ITx recipients with positive results. For patients demonstrating symptoms at the time of recovery of adenovirus, high stool output, alone or in the presence of fever, were the most common symptoms found in these patients [24, 25]. While one report found no difference in all cause mortality rates for pediatric ITx recipients with and without adenovirus infection [33], mortality attributable to adenovirus was reported in the three series [25, 26, 34]. Adenovirus can also present as an invasive disease with risk factors including failure to clear virus, isolating, virus from more than one site and intensified immunosuppression.

34.4 Diagnosis

Presumptively diagnosing adenovirus infection after transplantation can be difficult. Typical manifestations seen in patients with disease due to adenovirus, such as fever, hepatitis, or pneumonitis may represent infection with adenovirus but can also be from a number of other pathogens, and also can be seen with rejection. Accordingly, the presence of high-grade fevers and symptoms suggestive of infection should prompt a diagnostic evaluation aimed at identifying all such possibilities. Adenovirus can be found through the use of antigen detection, culture, nucleotide amplification tests, or histopathology [1, 2, 39]. Rapid antigen detection kits are commercially available but their sensitivity and specificity in the SOT population is unstudied. While traditional viral cultures are available, they are used less frequently in the era of shell vial assays and nucleic acid–based real-time polymerase chain reaction (PCR) assays. These latter tests permit more rapid diagnosis and increase sensitivity for adenovirus [1, 39]. Unfortunately, finding of a positive test for adenovirus is not definitively diagnostic of symptomatic disease as adenovirus can be asymptomatically shed for prolonged periods of time in urine, upper respiratory secretions, and stool [2, 39]. Accordingly, recovery of adenovirus should always prompt an effort to identify any additional or alternate potential explanations for symptoms present concurrently. Detection of adenovirus at two or more sites has been found to be predictive of invasive disease in bone marrow transplant (BMT) recipients [3, 39]; this has also been observed after pediatric LT [8]. Nucleic acid amplification can use quantitative or qualitative PCR for detection adenovirus in multiple specimen types. The relative sensitivity of PCR assays is dependent on the specimen, the primers used, and the characteristics of the method; as such, not all nucleic acid tests will detect all adenovirus serotypes. Preliminary experience suggests that quantitative nucleic acid detection of adenovirus genomes in blood appears to have prognostic significance and can be used to monitor response to therapy [40–45].

Given the concerns regarding sensitivity and specificity for the above-mentioned diagnostic tests, it is not surprising that histologic evaluation remains the gold standard for the diagnosis of invasive adenoviral disease [1, 2, 39]. Adenoviral infection is associated with a typical cytopathic inclusion ("smudge cells"). The presence of the virus within tissue may also be identified through the use of confirmatory histochemical stains. Most experts would consider the presence of adenovirus in a histologic specimen in association with a compatible clinical syndrome to be diagnostic of disease attributable to adenovirus.

34.5 Treatment

There currently is no FDA-approved therapy for the treatment of adenoviral infection. For some patients with more limited disease, such as kidney transplant recipients with hemorrhagic cystitis or interstitial nephritis, management can often be accomplished with reduction of immunosuppression alone. In general, all patients with adenovirus infection should have immunosuppression reduced to speed recovery of infection along with general supportive care. Although the role of antiviral agents is unproven, therapy has resulted in reduced progression of disease and reduced mortality in patients with disseminated infection or those with more severe manifestations of infection. A number of agents show variable in vitro activity against adenoviruses. However, interpretation of these results is limited by the use of different techniques, cell lines, and viral isolates [39, 46, 47]. There are case reports and series describing the use of cidofovir [30, 46–51], ribavirin [44, 52–55, 74], and ganciclovir [29, 56–59] in the treatment of adenoviral infection after SOT or BMT. Ribavirin use is associated with significant toxicity [39, 41, 46], and its activity against adenovirus may be limited to subtype C viruses [60]. To date, ribavirin has not been documented to reduce viral titers in monitored patients [8, 44, 60], and most experts recommend against the use of ribavirin in the treatment of adenovirus [3, 39]. Likewise, data to support the use of ganciclovir for the treatment of adenovirus is limited and is generally not used for the treatment of severe infections.

Of all proposed antiviral agents for adenovirus, cidofovir and its lipid ester, brincidofovir (CMX001) have the best evidence to support its use [3, 46, 48–51, 61–63]. In studies using molecular monitoring of infected patients, most, but not all, patients demonstrated a virologic response to cidofovir therapy, which correlated with clinical improvement [42, 43]. Failure to have a one log or greater decline in viral loads in the first 2 weeks of treatment was associated with progression of viremia and death secondary to symptomatic disease [42]. Typically, one of two regimens of cidofovir has been used for the management of adenoviral disease: 5 mg/kg q1–2 weeks or 1 mg/kg three times a week [39, 41, 49–51, 53]. Patients are usually hydrated before and after treatment and receive probenecid with doses to prevent nephrotoxicity. Although the regimen of 1 mg/kg three times per week is associated with less nephrotoxicity [49], the efficacies of the two regimens have not been directly compared. Additionally, the 1 mg/kg three times per week regimen is associated with

breakthrough CMV and herpes simplex virus (HSV) infections [64, 65]. More recently, brincidofovir (CMX001) has been demonstrated to have excellent oral bioavailability with improved in vitro and in vivo activity against adenovirus (5 to greater than 2500-fold more potent against adenovirus, in terms of IC50 values, than the unmodified parent compound) [66–68]. Further, brincidofovir is associated with no significant nephrotoxicity or bone marrow toxicity, although dose-limiting diarrhea has been described [61, 63, 66, 67]. In a prospective study of brincidofovir for the management of adenovirus in 13 immunocompromised patients with adenovirus, eight patients had a ≥ 1 $\log_{10}$ drop in viral load after the first week of therapy and nine had demonstrated a virologic response by week 8. Patients with a virologic response had a longer survival than those without a response (median 196 days versus 54.5 days; P = .04) [61]. A phase 3, open label study of brincidofovir is currently studying the safety and efficacy of this novel compound in patients with adenovirus (clinicaltrials.gov identifier NCT02087306).

The use of antibody preparations, including polyclonal immunoglobulin with high titers against respiratory syncytial virus (Respigam, MedImmune, Inc. Gaithersburg, MD), have been used in a few cases with unclear benefit [51] but has biologic plausibility [69, 75].

Finally, the use of adoptive immunotherapy has been reported for the treatment of life-threatening adenoviral infections after T-cell-depleted BMT [70, 71]. However, efforts to apply similar strategies for EBV in SOT recipients have yet to be proven successful. Accordingly, while the use of this strategy for BMT recipients may become a therapeutic option, it is not applicable to SOT recipients at this time. Nonetheless, large-scale production of a panel of adenovirus-specific and multivirus-specific T cells has been demonstrated and shows promise as an emerging therapy [72, 73].

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35 Human Polyomavirus and Papillomavirus Infection and Disease Posttransplant

Hans H. Hirsch

35.1 Introduction

Infectious complications are accentuated in transplant patients as the result of the immunosuppression needed to disable alloimmune reactions between graft and host such as graft rejection or graft-versus-host disease. For viral infections, the intensity of immunosuppression not only correlates with a higher frequency of infectious episodes, but also with an increased level and duration of viral replication during such episodes, and consequently a greater likelihood of organ manifestations [1–5]. Moreover, virus-induced organ pathology is favored when virus-infected cells and immune effectors meet in an allogeneic constellation [1]. Thus, after solid organ transplantation (SOT), liver, lung, and kidney transplants appear to be more prone to complications from hepatotropic, pulmotropic, and nephrotropic viruses, respectively, whereas after allogeneic hematopoietic stem cell transplantation (HCT), excess pathology can arise in virtually every organ targeted by viral infection. Thus, in addition to immunosuppression, virus replication inside infected host cells is less effectively controlled if viral epitopes are presented in the context of a MHC-I versus T-cell receptor mismatch. Moreover, mounting an antiviral immune response in this allogeneic constellation bears an increased risk for allosensitization, chronic inflammation, and smoldering viral replication, which together or alone may be correlates of the so-called "indirect" viral effects [6, 7]. This rule of thumb also applies to human papillomavirus (HPV) and human polyomavirus (HPyV) infections, which show an aggravated clinical course in transplant recipients. HPV and HPyV share some similarities in morphology being nonenveloped icosahedral particles of 40–55 nm in diameter, in host range being restricted to the humans as well as in their oncogenic potential. Since 2002, however, HPV and HPyV are no longer grouped together as *papova* viruses, but are now recognized as distinct virus families called *papillomaviridae* with 39 genera and *polyomaviridae* with 4 genera including more than 70 species [8–10].

35.2 Human Polyomavirus (HPyV) Infection

The discovery of PyV dates back to the 1950s with the identification of an infectious agent causing multiple tumors in newborn mice, hence providing the name (Greek: poly, multiple; -oma, tumor) [11]. Since then, PyVs have been detected in a variety of vertebrates including rodents, birds, cattle, monkeys, and primates. Today, 13 PyVs has been detected in human specimens, which differ in age-dependent seroprevalence rates, associated pathology, and presumably host cell tropism (Table 35-1) [12, 13].

PyV are fairly resistant to environmental inactivation and endure temperatures of up to 50 °C for 1 h [8, 14]. The PyV genome is a circular double-stranded DNA of about 5100 base pairs containing a short noncoding control region (NCCR) of 400 bp bearing the origin of viral genome replication as well as promoter/enhancer elements controlling expression of the early viral gene region (EVGR) and the late viral gene region (LVGR) in opposite directions from the NCCR. The EVGR encodes the regulatory the small T-antigen (sTag) and large T antigen (LTag), which is highly conserved and often targeted in immunohistochemical diagnosis of PyV disease. The LVGR encodes the viral capsid proteins Vp1, Vp2, Vp3, as well as the small regulatory agnoprotein. Human PyV (HPyV) genomes are 50–85 % homologous both at the nucleic acid and the amino acid level. These similarities need to be taken into account when using molecular and immunologic assays for research and diagnostics [15, 16].

Based on serological studies, primary BKPyV transmission seems to occur efficiently in children below 10 years of age, reaching an IgG seroprevalence of at least 90 % by early adolescence [17–20]. In healthy blood donors, the average seroprevalence is 82 % and declines with increasing age [19, 21]. In dialysis patients, lower antibody titers have been

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ABLE 35-1. Human polyomavirus (HPyV) infections

Polyomavirus	Recommended abbreviation	Seroprevalence (%)	Persistent infection	Disease immunocompetent	Immunocompromised	Highest risk
BK polyomavirus [a](patient initials)	BKPyV	80–95	Kidney	Cystitis Encephalitis? Prostate cancer?	PyV-associated nephropathy, Ureteric stenosis, Urothelial carcinoma, PyV-associated hemorrhagic cystitis	Kidney transplants (rarely other SOT, HCT) Allogeneic HCT (rarely SOT)
JC polyomavirus [a](patient initials)	JCPyV	35–60	Kidney Central nervous system? Lymphocyte?	Tonsillitis? Colon cancer?	Progressive multifocal encephalopathy, Granule cell neuronopathy Meningitis, Encephalitis PyV-associated nephropathy	HIV-AIDS, Multiple sclerosis patients treated with natalizumab (allogeneic HCT, SOT)
KI polyomavirus (Karolinska Institute)	KIPyV	60–80	Unknown	Tonsillitis? Bronchitis?	Pneumonia? Sputum production?	Allogeneic HCT?
WU polyomavirus (Washington University)	WUPyV	50–70	Unknown	Bronchitis?	Pneumonia? Wheezing?	Allogeneic HCT?
Merkel cell polyomavirus	MCPyV	50–90	Skin (tonsils?)	Unknown	Merkel cell carcinoma	Older age, sun exposure, immunosuppressed (Kidney, heart, and lung transplants)
Trichodysplasia spinulosa polyomavirus	TSPyV	70	Skin	Unknown	Trichodysplasia spinulosa (Pilomatrix dysplasia, eye brow alopecia)	Kidney transplant (other SOT, leukemias)
Human polyomavirus 6	HPyV6	35–95	Skin	Unknown	Unknown	
Human polyomavirus 7	HPyV7	35–85	Skin	Unknown	Proliferative keratinopathy, Puritic plaques	Lung transplant
Human polyomavirus 9	HPyV9	20–69	Gut?	Unknown	Unknown	Kidney transplant
Human polyomavirus 10 (Malawi, MW-; Mexico, MX-)	HPyV10 (MWPyV, MXPyV)	25–70	Gut?	Diarrhea?	Unknown	
Human polyomavirus 11 (Saint Louis, STL-)	HPyV11 (STLPyV)	20–90	Gut?	Diarrhea?	Unknown	
Human polyomavirus 12	HPyV12	Unknown	Gut?	Diarrhea?	Unknown	
Human polyomavirus 13 (New Jersey, NJ-)	HPyV13 (NJHPyV13)	Unknown	Skin, Gut?	Unknown	Proliferative keratinopathy vasculopathy, myopathy	Kidney-pancreas transplant
Simian virus 40	SV40	<5 %	Unknown (kidney?)	Mesothelioma? Lymphoma? Glomerulosclerosis? Glioblastoma?	PyV-associated multifocal encephalopathy? Polyomavirus-associated nephropathy?	Uncertain

[a]Patient name should not be identified.

observed [22]. By contrast, and despite a certain degree of cross-protection [19, 23], JCPyV seroprevalence continues to increase during adult life to an average of 58 % indicating continued exposure and primary infection during adult life [18, 19, 24]. In HIV-seropositive patients, JCPyV IgG have been reported in more than 90 % suggesting a risk for increased transmission associated with HIV infection [25, 26]. For KIPyV, WUPyV, and MCPyV, slightly lower seroprevalence, though similar age dependence, has been determined (Table 35-1) [20, 27, 28]. For SV40, seropositivity seems to be <5 % arguing against a significant SV40 circulation in human populations despite documented vaccine contaminations in the 1960s and possibly ongoing exposure in zoological parks and animal facilities.

Primary HPyV infections have not been linked to specific symptoms or signs indicating that they are largely subclinical or run an unspecific, e.g., flu-like course. Transmission presumably occurs via mucosal surfaces exposed through ingestion, inhalation, or inoculation of virion-contaminated fluids. For BKPyV and JCPyV, kidney and urinary tract have been identified as the principle sites of persistence. Importantly, reactivation and asymptomatic urinary shedding of BKPyV and JCPyV is observed in healthy blood donors at median rates of 7–10 % and 19–33 %, with urine viral loads of 3.5 and 4.6 $\log_{10}$ genome equivalents per mL (Geq/mL), respectively [19, 29]. Thus, the detection of BKPyV or JCPyV in the urine per se is not an indicator for disease. For KIPyV and WUPyV, respiratory transmission is strongly implicated, but the specifics are still unresolved including aspects of latency, reactivation and the high rate of coinfection with other viruses [30, 31]. WUPyV DNA was identified in 43 samples obtained from 2135 patients with acute respiratory tract infections. Of those, 31 were also positive for other respiratory viruses [32]. Screening of immunocompetent individuals with respiratory tract infection detected KIPyV and WUPyV in 1–7 % without pronounced seasonality, but preferably in pediatric patients [33, 34]. Using KIPyV VP1-specific PCR, positive results were obtained in 1 % (6 of 637) nasopharyngeal aspirates from patients with respiratory tract disease and 0.5 % (1 of 192) stool samples from patients with gastroenteritis [35]. KIPyV could not be detected in urine, serum, whole blood or isolated leukocytes. MCPyV, HPyV6, and HPyV7 have been detected in a relatively large proportion in skin samples from otherwise healthy individuals [36–38]. Thus, the MCPyV-associated skin carcinoma is likely to require cofactors besides MCPyV infection such as failing immune control, older age, and sun exposure, but possibly also coinfection and interaction with other polyomaviruses [12]. At this point, the clinical significance of HPyV infection in immunocompetent individuals is still unclear.

In patients with impaired immunity, HPyV replication appears to be relaxed, and the prevalence of BKPyV replication and urinary shedding significantly increases to 50–80 % [39, 40]. High-level BKPyV viruria defined as decoy cell shedding or the equivalent of >7 $\log_{10}$ Geq/mL is observed in more than half of these patients [39, 41–47]. Molecular and kinetic data from kidney transplant patients support the model of BKPyV latency and reactivation in renal tubular epithelial cells followed by amplification in the urothelial cell compartment [43]. The respective increases in JCPyV shedding are less pronounced in immunodeficient populations [19, 25, 41, 48], and JCPyV viremia remains an exception [48–50]. For KIPyV and WUPyV, tenfold higher detection rates have been reported in immunosuppressed patients [51–53]. In fact, all significant, pathologically defined HPyV diseases have occurred in individuals with impaired immune functions. Despite the environmental stability and the high HPyV loads in urine and respiratory secretions, there is so far only one report of nosocomial transmission clusters, opening the question about specific infection-control measures for patients at high risk [54], which are currently not deemed necessary without further evidence [55].

35.3 HPyV Diseases in Transplant Patients

The key HPyV diseases posttransplant are PyV-associated nephropathy (PyVAN), PyV-associated hemorrhagic cystitis (PyVHC), and PyV-associated progressive multifocal leukoencephalopathy (PML) (Table 35-1). PyVAN and PyVHC are primarily associated with BKPyV, but a minority of cases are implicating JCPyV [48, 50, 56, 57]. Conversely, progressive multifocal leukoencephalopathy is primarily mediated by JCPyV [24], but a few cases may be caused by BKPyV [58]. In addition, a variety of gastrointestinal or typically proliferative skin disorders have been linked to other HPyVs causing diarrhea, or Merkel cell carcinoma, *Trichodysplasia spinulosa*, and pruritic keratinocyte plaques (Table 35-1).

35.4 BKPyV

35.4.1 Polyomavirus-Associated Nephropathy (PyVAN)

The incidence of PyVAN ranges from 1–10 % in kidney transplant recipients with a peak in the first 6 months posttransplant [39]. PyVAN is typically asymptomatic with no other sign than progressive renal allograft failure. Testing for BKPyV replication demonstrates decoy cells in urine cytology, urine viral loads of >7 $\log_{10}$ Geq/mL and, at the same time, detectable plasma BKPyV loads (probable PyVAN) [39, 43, 59, 60]. Plasma BKPyV loads have been used as a surrogate marker of the disease, which becomes more likely with increasing duration and level, e.g., above 4 $\log_{10}$ Geq/mL (presumptive PyVAN). The definitive diagnosis of proven PyVAN requires allograft tissue showing typical

cytopathic changes in renal tubular epithelial cells, which are confirmed by immunohistochemistry for large T-antigen, Vp1 or agnoprotein expression or by in situ hybridization [61–64]. Immunohistochemistry has mostly relied on cross-reacting antibodies raised against the SV40 LTag. Although BKPyV causes more than 95 % of cases, this cross-reactivity is actually advantageous since rare cases of JCPyV-mediated PyVAN are detected as well [48, 50]. Classification of the histological features has been recommended in order to predict the risk of subsequent graft loss and enhance the comparability of clinical studies (Table 35-2; for details, see [59, 60]).

The limitation of the histological diagnosis resides in the (multi-)focal nature of PyVAN, causing false negative biopsy results in 10–30 %, which may be even higher early in the disease when serum creatinine concentration is still at baseline levels [59, 63]. Also, the distinction of PyVAN from acute interstitial rejection is difficult and may require adjunct histological evaluation of vascular rejection including staining for C4d [13, 60]. The differential diagnosis of tubular cytopathology with intranuclear inclusion and interstitial nephritis/tubulitis includes adenovirus and cytomegalovirus nephritis. Of note, high-level BKPyV viruria with urine loads of >7 $\log_{10}$ Geq/mL are found 30–60 % of kidney transplant patients and precedes by approximately 6 weeks the detection of plasma BKPyV loads in a subpopulation of patients (10–20 %) and PyVAN [39].

The risk factors of PyVAN include donor characteristics (such as female gender, deceased donation, ischemia–reperfusion injury, high BKPyV-specific antibody titers as a marker for recent exposure, HLA-mismatches), recipient characteristics (such as older age, male gender, low or absent BKPyV-specific T-cell activity), and posttransplant factors such as acute rejection and antirejection treatment with high cumulative steroids, depleting anti-thymocyte globulin, intensified immunosuppressive rescue protocols, and tacrolimus–mycophenolate–prednisone combinations [39, 47, 59, 65–67]. Though multiple and diverse, these entities may indeed reflect complementing factors in PyVAN pathogenesis, the central feature being a disrupted balance between BKPyV replication in renal tubular epithelial cells and BKPyV-specific cellular immune control [68, 69]. Studies of plasma BKPyV load kinetics in kidney transplant patients undergoing surgical graft removal or clearing BKPyV replication following reduced immunosuppression indicated a short BKPyV half-life of 2–12 h in plasma as well as in urine [43]. Thus, stable steady-state levels actually result from a >99 % replacement of the plasma virus load every day [43]. The resulting cytopathic loss can be estimated as in the order of 1–10 million tubular epithelial cells leading to extensive denudation of the tubular epithelial cell layer with necrosis, urine leakage, ensuing inflammation, and eventually tubular atrophy and fibrosis (Figure 35-1). By contrast, the multilayered urothelial cell layer remains largely intact in kidney transplant patients despite high urine viral loads and hence without concomitant denudation, inflammation, and clinical signs [43]. Thus, BKPyV viruria and viremia remain the most consistent marker for screening, early intervention, and monitoring outcome. In view of the limited sensitivity of allograft biopsies and the higher specificity of plasma BKPyV loads compared to urine BKPyV loads, a diagnosis of probable and presumptive PyVAN has been used to guide preemptive treatment (Table 35-2).

TABLE 35-2. PyVAN diagnosis

- Possible PyVAN
  - High-level PyV replication
    Urine viral load >7 $\log_{10}$ Geq/mL, mostly BKPyV, rarely JCPyV; decoy cells
  - Plasma BKPyV load not tested or undetectable
- Probable PyVAN
  - High-level PyV replication
    Urine viral load >7 log Geq/mL mostly BKPyV, rarely JCPyV; decoy cells, PyV particles in three-dimensional aggregates (“haufen”) by electron microscopy of urine, BKPyV VP1 mRNA > 6 $\log_{10}$ copies/ng total RNA
  - Plasma BKPyV load detectable
- Presumptive PyVAN (“laboratory-confirmed”)
  - Plasma BKPyV load increasing to >4 $\log_{10}$ Geq/mL for >3 weeks
- Proven PyVAN
  - PyVAN-A: Mild viral cytopathic changes in ≤25 % of tubules with minimal interstitial inflammation, tubular atrophy, and interstitial fibrosis <10 % of the biopsy core. Risk of kidney graft loss <10 %
  - PyVAN-B: Variable viral cytopathic changes in 11–>50 % of tubules, significant inflammatory infiltrates with tubulitis, but only mild tubular atrophy and fibrosis in ≤25 % of the biopsy core
    PyVAN-B1 moderate interstitial inflammation in 11–25 % of biopsy: allograft function slightly impaired, risk of graft loss 25 %
    PyVAN-B2 significant interstitial inflammation in 26–50 % of biopsy: allograft significantly impaired, risk of graft loss 50 %
    PyVAN-B3 extensive interstitial inflammation in >50 % of biopsy: allograft significantly impaired, risk of graft loss >75 %
  - PyVAN-C: Moderate to severe tubular atrophy and interstitial fibrosis affecting >25 % of renal parenchyma, viral cytopathic changes variable and inflammatory infiltrates variable, ranging from <10 % to >50 %: Allograft function significantly impaired, progressive failure, and risk of kidney graft loss >80 %

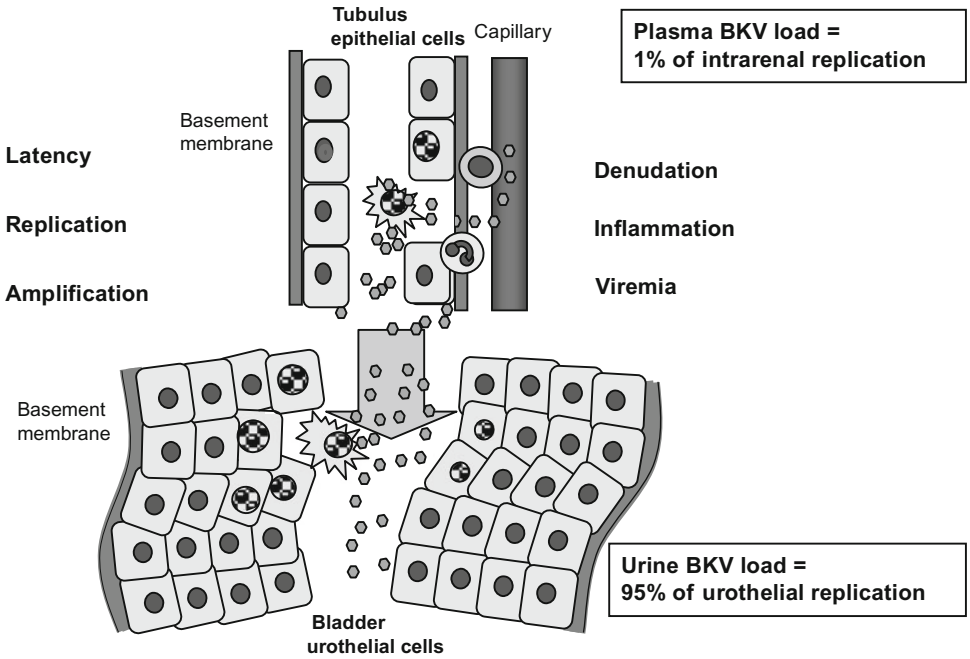

FIGURE 35-1. PyV replication and pathology in nephropathy.

Screening for BKPyV replication identifies kidney transplant patients before extensive tissue damage and irreversible loss of renal graft function has occurred [39, 70, 71]. Protocol biopsies are suboptimal for this purpose since approximately one third of cases are missed, which then run a worse course [70]. Testing urine for BKPyV replication by urine cytology (decoy cells) or quantitative PCR allows to rule out PVAN with a high negative predictive value of >95 %, but the positive predictive value is low. Patients with high-level urinary BKPyV replication should be tested for plasma BKPyV DNA load. If plasma BKPyV loads are positive and increasing to >4 $\log_{10}$ Geq/mL, reducing immunosuppression should be considered even in the absence of a positive histology [59, 72–76]. Until an internationally accepted calibrator for standardizing BKPyV loads has been implemented across laboratories, the relevance of this threshold has been debated. Unlike the experience with cytomegalovirus loads, however, external quality assurance programs in Europe indicate that 95 % of the 120 participating laboratories deviate less than 0.5 $\log_{10}$ Geq/mL from one another. At least six prospective studies supported the feasibility and safety of this preemptive approach without progression to PyVAN and without an excess of acute rejection episodes [72, 76, 77]. Currently, a minimal screening is recommended in 3 monthly intervals during the first 2 years posttransplant, or when an allograft biopsy is performed [13, 59, 60]. This basic approach has been expanded to monthly screening up to month 6 posttransplant and then 3 monthly in some centers. Modeling data suggest a benefit at PyVAN rates of >2.1 % when assuming adverse events in 10 % of cases [78]. However, screening may be beneficial already at lower rates given the good outcomes in dedicated centers [59, 72–76].

The treatment of PyVAN relies on timely reduction of immunosuppression [60]. Specific antivirals are lacking, but have been used as adjunct therapy [79]. There are no clinical studies comparing one strategy with another, but there is general consensus, that PyVAN should be treated by reducing tacrolimus to trough levels to <6 ng/mL (3 ng to 5 ng/mL), mycophenolate mofetil dosing be reduced to less than 1000 mg per day (or equivalent) or even discontinued, and prednisone reduced to 10 mg or less per day [59]. For cyclosporine, trough levels of 100–125 ng/mL have been proposed. Currently, no recommendation can be made whether or not calcineurin inhibitors or antiproliferative drugs should be reduced first. In the preemptive situation, both approaches have been successful [13]. Studies of BKPyV-specific immunity in kidney transplant patients and in vitro studies using BKPyV-specific cellular immunity indicate that the calcineurin inhibitor concentration is most critical and that for patients with definitive PyVAN, reducing calcineurin inhibitors might be more effective as a first step [80]. Biweekly plasma BKPyV loads are commonly used as a surrogate marker for tubular epithelial cell replication. Plasma BKPyV loads respond earlier than urine BKPyV loads, and a decline of >2 $\log_{10}$ Geq/mL is associated with the emergence of BKPyV-specific T-cell activity in peripheral blood [69, 81, 82]. Clearance typically takes 8–12 weeks. Some centers add low-dose intravenous cidofovir at 0.25 mg to 1.0 mg/kg bodyweight to this intervention (cave renal and ocular toxicity; [79]). In vitro studies indicate that concentrations of 40 µg/mL are needed for a 90 % inhibition [83], but peak serum concentrations of only 5 µg/mL have been observed in vivo [84]. Accordingly, several studies have been disappointing [85, 86]. Similarly, the use of fluoroquinolones for prohylaxis or treatment has not been effective [86, 87].

Switching from mycophenolate to leflunomide, a pyrimidine synthesis inhibitor used for treating rheumatoid arthritis, has been advocated because of its presumed virostatic properties [88, 89]. Similar to cidofovir, randomized controlled trials for leflunomide efficacy are lacking. Since leflunomide must be viewed as a less potent immunosuppressant than mycophenolates, it is currently impossible to attribute the clearing of BKPyV replication and nephropathy to improving BKPyV-specific immunity or to a combined effect with antivirals [90]. More recently, switching to mTOR-inhibitors has been proposed as a possible intervention with a direct inhibitory effect on BKPyV replication, while preserving BKPyV-specific immune activation [80, 91–94].

Retransplantation after allograft loss due to PyVAN can be considered, but recovery of BKPyV-specific immunity should be taken into account [95, 96]. Clearance of plasma BKPyV or at least a decline of >2 log Geq/mL may be used as an indicator [81]. Surgical removal of the affected first graft is not a prerequisite, but this may be advisable for preemptive retransplantation to avoid rapid reinfection of the new graft and to allow unambiguous attribution of BKPyV screening assays to the new transplant. No specific recommendation for immunosuppression has been made other than targeting the lower end of normal. Whether or not induction treatment for retransplantation increases the risk of PyVAN recurrence is not known [95, 96].

PyVAN has been sporadically diagnosed in autologous kidneys in non-renal SOT or HCT despite similar or even more intense immunosuppression suggesting that factors specific to kidney transplantation must be operating [13, 97–101]. Most patients were identified with advanced inflammatory PyVAN-B stage and progressed to terminal renal failure with dialysis-dependence despite reducing immunosuppression and/or addition of cidofovir. Where examined, significant viremia and viruria was noted in most of these patients as reported for kidney transplant recipients. Larger studies indicate however that PyVAN is an exceptional complication in non-renal SOT. Thus, non-renal SOT and allogeneic HCT with creeping serum creatinine concentrations, PyVAN should be considered in the differential diagnosis and testing for BKPyV viremia be performed, but universal screening for BKPyV replication is presently not warranted [41, 102, 103]. Less than 5 % of PyVAN may be caused by JCPyV. The clinical presentation of JCPyV-PyVAN is indistinguishable from BKPyV-PyVAN, with comparable cytopathic changes in renal tubular epithelial cells and corresponding signs of inflammation [48, 50, 56]. Thus, the diagnosis of JCPyV-PyVAN requires high-level JCPyV loads in urine (>7 $\log_{10}$ Geq/mL), absence of BKPyV replication, compatible histopathology changes (PyVAN-A, -B, or -C), and the exclusive detection of JCPyV in the allograft, e.g., by PCR. The NCCR in JCPyVAN is typically not rearranged unlike the JCPyV genomes in PML. Of note, JCPyV viremia is rare and usually of low viral load and cannot be used as a sensitive marker for screening and monitoring. With some exceptions of late diagnosis, kidney allograft function was often preserved and histological clearance was observed following reduced immunosuppression [104]. Urine JCPyV loads declined, but remained persistently detectable at levels similar to immunocompetent individuals [19, 48, 50].

35.4.2 Polyomavirus-Associated Hemorrhagic Cystitis (PyVHC)

PyVHC complicates 5–15 % of allogeneic HCT around 3–6 weeks posttransplant (late onset) and is associated with higher costs in the early transplant period [105] and an overall poorer survival [106, 107]. Most patients are hematologically stable and have engrafted. However, cystitis with urinary urgency and often disabling pain, and macrohematuria with clots require hospital admission and dedicated inpatient care with analgesia, forced diuresis, bladder irrigation, and urologic intervention for bleeding and relief of postrenal failure. PyVHC should be distinguished from other causes of hematuria and/or cystitis [108, 109]. This may be sometimes difficult because of overlapping patient characteristics and risk factors [110]. Hemorrhagic cystitis with severe hematuria (grade II–IV) has been reported in up to 5 % of 1906 patients after HCT by Seber et al. [111] with allogeneic HCT, patient 10–30 years of age, cyclophosphamide, busulfan, and graft-versus-host disease [111]. This included early-onset hemorrhagic cystitis which occurs prior to engraftment and is largely attributed to urotoxic conditioning regimens with cyclophosphamide, ifosfamide, busulfan, and/or TBI and [40, 108, 112, 113]. It is important to note that BKPyV viruria reaching high viral loads of >7 $\log_{10}$ Geq/mL is observed in 50–80 % of HCT patients, but less than one fifth develop PyVHC [40, 112, 114]. Clearly, high-level BKPyV replication with viruria is necessary, but not sufficient for the diagnosis of PyVHC. Similarly, hematuria is quite frequent after allogeneic HCT [40] and may be due to different causes such as thrombocytopenia, coagulation disorders, and graft-versus-host disease.

The diagnosis of PyVHC requires the triad of cystitis, hematuria (grade II or more), and high-level BKPyV replication with urine loads of >7 $\log_{10}$ Geq/mL (Table 35-3). In addition, other diagnoses should be excluded including other infections and bleeding disorders [40, 54, 79, 112, 115]. Cystoscopy may be indicated for local hemostasis and clot removal to treat post-renal failure, but is rarely performed for a histological diagnosis because of bleeding complications. Similarly, imaging studies such as ultrasound, computer tomography, or magnetic resonance imaging searching for posttrenal obstruction may reveal thickened bladder and ureter walls, but are not required for the diagnosis [116]. Urine BKPyV loads may be higher (e.g., greater than 9 $\log_{10}$ Geq/mL) in cases with PyVHC than in asymptomatic patients, but the levels frequently overlap [45, 46, 117–119]. BKPyV viremia has been associated with an increased risk of PVHC.

TABLE 35-3. Polyomavirus-associated hemorrhagic cystitis (PyVHC) Diagnosis

Triad of	
1. Clinical signs of cystitis	
(a) Dysuria, urge, frequency, lower abdominal discomfort	
2. Hematuria of Grade II and higher	
(a) *Grade I* microscopic hematuria (>100 erythrocytes per high-power field)	
(b) *Grade II* macrohematuria	
(c) *Grade III* macrohematuria with clots	
(d) *Grade IV* macrohematuria with urinary obstruction and post-renal failure	
3. Laboratory markers of high-level PyV replication in urine	
(a) Urine viral load >7 $\log_{10}$ Geq/mL	
(b) Plasma viral load detectable, often >4 $\log_{10}$ Geq/mL	
(mostly BKPyV, rarely JCPyV >7 $\log_{10}$ Geq/mL; or decoy cells)	
• Exclude major contribution of other diagnosis or factors	
Urotoxic	Early-onset HC
Hematological	Bleeding disorder, low platelets, graft-versus-host disease
Infectious	Bacteria; virus, e.g., cytomegalovirus, adenovirus; fungus; parasite
Malignant	Local or metastasizing (e.g., urothelial carcinoma)
Mechanical	Catheter, urologic intervention

BKPyV loads were been detected in sera of one-third of HSCT recipients in the first 100 days after HSCT and sustained serum BKPyV loads of >4 $\log_{10}$ Geq/mL were significantly associated with PyVHC [54, 106, 120, 121]. At present, validation of urine BKPyV load kinetics or plasma BKPyV is lacking regarding their use for diagnosis and for their potential use to trigger preemptive interventions.

Risk factors for PyVHC include allogeneic HCT, acute GvHD, unrelated donor, myeloablative conditioning, cord blood HCT, and a higher BKPyV recipient antibody titer before HCT [106, 109, 118, 122]. In fact, BKPyV replication represents secondary reactivation in seropositive patients, even in children [123], but the role of the mismatch of seronegative donor (D−)/seropositive recipient (R+) is unknown. However, in some cases of presumed nosocomial BKPyV transmission, pediatric allogeneic HCT recipients with low or absent BKPyV IgG developed PyVHC, and the disease only resolved when BKPyV antibody responses increased, independent of systemic or intravesical cidofovir treatment. Nevertheless, there is currently not enough evidence to support routine testing of HCT recipients or donors for the presence of BKPyV-specific antibodies.

The pathogenesis of PyVHC is not well understood. Urotoxicity of the conditioning protocol, particularly by cyclophosphamide and ifosphamide, is known to activate local inflammation through oxygen radicals, TNFa, IL-1b, and NFkB signalling as well as outright DNA and cell damage of urothelial, submucosal and smooth muscle cells [124]. These factors also enhance BKPyV replication via transcription factor binding sites such as NFkB and AP1 contained in the BKPyV noncoding control region, while at the same time, BKPyV-specific immune control is ablated. Forced diuresis, mesna, and reduced intensity protocols decrease the overall urotoxicity of conditioning, but may still lead to significant local exposure, particularly in the bladder causing limited local urothelial damage with erosion, edema, vascular congestion, and impaired regeneration [125]. In such a structurally and functionally altered environment, high-level BKPyV replication may significantly add to the urothelial cytopathology which, in contrast to kidney transplant patients showing a similarly high cytopathic wear, leads to urothelial denudation, urine leakage, inflammation, and hematuria [43]. Inflammation may be further aggravated postengraftment due to an immune reconstitution inflammatory syndrome [126–128]. The role of local toxicity is consistent with the reduced rate of PyVHC after reduced intensity conditioning. The preferential manifestation of PyVHC in allogeneic HCT as compared to autologous HCT has suggested a contributory role of graft-versus-host disease. On the other hand, neutropenia is often shorter in autologous HCT. Thus, we postulate the following sequence of events (Figure 35-2): (1) Reactivation of BKPyV replication from latency in renal tubular epithelial cells; (2) Limited damage of the urothelial transitional cell layer by urotoxic conditioning; (3) High-level BKPyV replication in the urothelial cell layer through cell damage and regeneration and similar lack of antiviral immune control; (4) significant denudation, hemorrhage, and inflammation; and (5) Exacerbation upon recovery of immunity post-engraftment [43, 126, 127]. Local urothelial denudation and inflammation may also facilitate the leakage of BKPyV into the circulation thereby explaining the diagnostic potential of plasma BKPyV loads [120, 121, 129]. Despite being an attractive model of PyVHC to researches in the field [130], detailed studies are needed for validation and hopefully the development of rational therapies.

The treatment of PyVHC is unresolved despite an array of published case reports and a variety of approaches. The reported response rates are high, but are based on small uncontrolled case series only, the course of which cannot be distinguished from

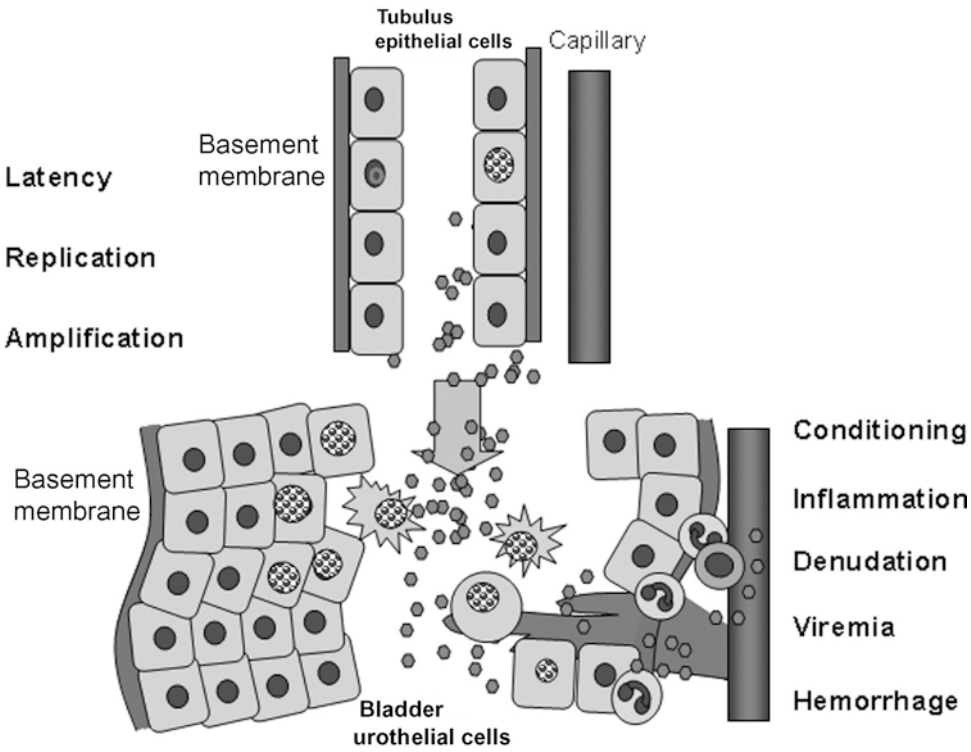

FIGURE 35-2. PyV replication and pathology in hemorrhagic cystitis.

spontaneous recovery. The mainstay of therapy is symptomatic with analgesia, hyperhydration, diuresis, and continuous bladder irrigation to prevent clot formation and urinary tract obstruction, platelet and erythrocyte substitution. Local treatment with alum, formalin or hyperbaric oxygen has been reported as beneficial. Urologic intervention may be performed for clot evacuation and for uncontrolled bleeding which may require cystectomy [131, 132]. Treatment with steroids, especially in patients with graft-versus-host disease may also alleviate the inflammatory component of PyVHC and reduce pain, but at the same time enhance BKPyV replication by direct activation through viral glucocorticoid response elements and impaired antiviral immune control. Reducing immunosuppression as proposed for treating PyVAN may increase inflammation. Thus, antiviral treatment seems urgently needed to break out of this double-blind pathology. Intravenous cidofovir as biweekly dosing of 5 mg/kg body weight together with probenicid or as lower dose of 0.25 mg, 0.5 mg or 1 mg/kg body weight given once to three times per week has been reported [79, 109, 133–135], but again, evidence from randomized controlled trials is lacking. In a retrospective survey in Europe, a presumed response to intravenous cidofovir was associated with younger age (<15 years), non-cord blood stem cell source, and total body irradiation as part of the conditioning regimen [134]. Intravesical cidofovir instillation (5 mg/kg in 60–100 mL saline) has been described as a therapy option, but was neither well tolerated nor effective in our experience. Fluoroquinolones such as ciprofloxacin or levofloxacin may inhibit BKPyV replication in vitro and reduce the peaking of urine BKPyV loads in HSCT patients in vivo, but randomized controlled studies are lacking [79], and a recent study in kidney transplant patients showed no effect [87]. In addition, fluorquinolones are already widely used as antibacterial prophylaxis during neutropenia and seemingly resistant BKPyV isolates have been reported [79, 136]. Thus, there is currently no established treatment for PyVHC other than symptomatic support with pain management, hyperhydration, and intravesical irrigation. Accordingly, no strategies regarding screening for urine BKPyV load or BKPyV viremia can be recommended. A summary of recent findings and recommendations have been presented at the sixth European conference for Infections in Leukemia [137].

PyVHC has been rarely reported in other patients including SOT patients, patients receiving chemotherapy or with HIV-AIDS receiving antiretroviral therapy, and even in one presumably immunocompetent individual with primary BKPyV infection [129, 138–142]. One case of PyVHC involving JCPyV has been reported in a patient with hereditary ataxia telangiectasia [143].

35.5 JCPyV

35.5.1 Progressive Multifocal Leukoencephalopathy (PML)

PML is a demyelinating disease of the central nervous system (CNS) that is primarily caused by lytic replication foci of JCPyV in oligodendrocytes. PML has been initially described in patients with chronic lymphatic leukemia and Hodgkin's lymphoma about 50 years ago [24, 144–146] and

PyV particles were seen by electron microscopy in a patient with chronic obstructive lung disease treated with corticosteroids [147]. PML is observed after HCT as well as after SOT. The incidence rates have not been determined, but must be lower than 0.1 % when extrapolating observations in HIV-AIDS populations where rates of up to 1 % and more have been reported [148, 149]. PML may occur more frequently and earlier after HCT, typically within the first year (range 1–20 months) as compared to a median of 23 months in SOT patients (range 1–132 months) [150–152]. It is postulated that this reflects the more pronounced immunodeficiency in HCT resulting from the underlying disease and its treatment as originally described [144] before as well as after HCT. Conversely, PML may occur rarely and only several years after kidney transplantation, with first symptoms appearing at a median of 37 months (range 5–120 months) [150–161]. While being consistent with lower immunosuppression after kidney transplantation, this may also indicate a cumulative risk from immunosuppression and JCPyV (re-) exposure. The case of a patient should be noted who developed PML 6 months after kidney retransplantation, but after having been immunosuppressed for his first transplant functioning for as long as 20 years [152].

PML is typically suspected when patients present with neurologic deficits (Table 35-4). If compatible findings in magnetic resonance imaging (MRI) are seen in an immunocompromised patient, the diagnosis becomes very likely. The deficits start frequently focally and compromise defined motor functions and sensory deficits particularly vision, as well as ataxia and are accompanied by initially subtle changes in mental status, memory, and speech [24]. Fits are rare HIV-AIDS, but may be more frequent with partial immunity in HIV-AIDS patients treated with antiretroviral therapy and in transplant patients. For a radiological diagnosis, magnetic resonance imaging (MRI) is preferred over computer tomography to reveal multiple lesions in subcortical areas, cerebellum and brain stem, which over time become confluent. These lesions are characterized in MRI by increased signal intensity in T2-weighted images without mass effect (improved signal contrast in fluid-attenuated inversion recovery and suppression of CSF signal intensity; hypointense in T1-weighted MRI) as well as signal in diffusion-weighted imaging. The growing experience from MRI surveyance of multiple sclerosis patients treated with the monoclonal integrin inhibitor natalizumab has lead to identification of presymptomatic lesions in some patients indicating a change in the diagnostic paradigm of PML. The key laboratory study is the detection of JCPyV DNA in cerebrospinal fluid (CSF) which has a high positive predictive value of >95 %, but only a limited negative predictive value of 50–80 % [162]. Therefore, other etiologies should be evaluated as well, including parvovirus B19, EBV, CMV, and HHV6 encephalitis/ventriculitis and toxoplasmic encephalitis. Other CSF parameters are not or only mildly altered including protein (<100 mg/dL) and cell counts, usually with normal lactate and glucose concentrations. The definitive diagnosis of proven PML requires the histological detection of enlarged oligodendrocytes with nuclear inclusions in the periphery of demyelination lesions, which stain positive for JCPyV by immunohistochemistry or in situ hybridization (Table 35-4). In addition, lipid-laden macrophages and enlarged multinucleated astrocytes are seen. Inflammatory cells are typically missing in early stages, but in HIV-AIDS patients treated with antiretroviral therapy, inflammatory infiltrates are increasingly noted which may indicate an immune reconstitution inflammatory syndrome (IRIS), as also seen especially after discontinuation of natalizumab combined with plasmapheresis. IRIS is associated with clinical deterioration and new contrast enhancement in MRI and even epileptic fits. Biopsies for histopathology studies are considered when JCPyV is not detected in CSF and/or the epidemiological risk is unclear, and/or the clinical or radiological presentation is atypical. The key differential diagnosis in transplant patients is calcineurin inhibitor-induced leukoencephalopathy (cyclosporine, tacrolimus), which may present with radiologically similar lesions. Also, demyelination due to chemo/radiotherapy, other viruses, acute limbic encephalitis, CNS lymphoma, toxoplasmic encephalitis, and graft-versus-host disease ought to be considered, while multiple sclerosis is unlikely in the transplant setting. The course

Table 35-4. Diagnosis of progressive multifocal leukoencephalopathy (PML)

- Probable
  - focal neurological deficits *plus*
  - MRI showing corresponding T1 hypointense, T2 hyperintense lesions in subcortex cerebellum and brain stem, no significant contrast enhancement, no mass effect
  - Patients with inherited, acquired, or iatrogenic immune dysfunction
- Presumptive ("laboratory-confirmed")
  - Probable criteria plus
  - JCPyV DNA in cerebrospinal fluid (CSF)
    or JCPyV-specific intrathecal antibody (JCPyV-specific IgG index >1.5)
- Histology-confirmed
  - JCPyV-positive immunohistochemistry or in situ hybridization in the periphery of demyelinated lesions in brain tissue (biopsy, autopsy)
- Significant contribution of other (coexisting) pathology excluded

of PML is followed clinically and by MRI. JCPyV load in CSF has been proposed as a marker of severity and treatment response, but requires repeat lumbar taps. With few exceptions [163], JCPyV load in plasma is mostly low or undetectable, and urine JCPyV load cannot be used for screening or diagnosis as it is unrelated to the overall course [164].

The risk factors of PML have not been defined in transplant patients, mostly because of the sporadic onset in diverse clinical backgrounds. In analogy to PML in HIV-AIDS, profound long-standing cellular immunodeficiency and insufficient intracerebral antiviral immune surveillance are viewed as the key pathological mechanism [165]. This would also account for the occurrence of PML in patients treated with anti-T-cell reagents such as natalizumab, efalizumab, or FTY720 used for treating autoimmune diseases such rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, or psoriasis [166]. In HIV-AIDS, the median CD4 cell count at PML diagnosis is typically below 100 per microliter (median 60), but cases with higher CD4 counts occur [149]. A detailed study of HIV-positive PML non-survivors indicated that the overall counts were not decisive, but selectively impaired JCPyV-specific T-cell responses [26]. In the study [26], a protective role of antibodies, and consequently intact B-cell repertoires, was suggested which may also explain the occurrence of PML cases after anti-CD20 depletion using rituximab [167]. However, the vast majority of PML cases after rituximab had also been treated with antiproliferative drugs [167]. Of the eight transplant patients diagnosed with PML after rituximab (four autologous HCT, three allogeneic HCT, one kidney), all seven HCT patients had received alkylating agents [167].

The pathogenesis of PML is characterized by the uncontrolled replication of JCPyV in oligodendrocytes. The multifocality of PML pathology is reminiscent of PyVAN and suggests hematogenous spread. Expanding lesions reflect local cell to cell spread. However, it is not resolved whether or not JCPyV reaches the central nervous system during a primary infection and is later reactivated in the brain, or whether PML is the result of secondary reactivation in the periphery reaching the brain via lymphocytes [168]. The increasing JCPyV seroprevalence with age argues that significant JCPyV exposure occurs during adult life, which as primary or secondary replication could be the starting point of PML in severely immunocompromised individuals [19, 24]. A recent report of primary JCPyV infection and PyVAN argues that this can be case at times [50].

The treatment of PML is hampered by the lack of effective antivirals. Cidofovir and cytarabine have been explored in HIV-AIDS patients without convincing results [169–171]. Other attempts have explored serotonin uptake inhibitors (risperidone, mirtazipine), and mefloquine and chlorpromazine have been shown in vitro to interfere with JCPyV infection via the blockade of the 5-HT2a receptor or endocytosis, respectively [172]. The success of combination antiretroviral therapy in HIV-AIDS patients with PML indicates that recovering JCPyV-specific immunity must be an integral component of treatment [149]. However, discontinued immunosuppression may put transplant patients at risk of organ- and life-compromising alloimmune reactions, the only exception being kidney transplantation where organ function can be replaced by dialysis. It is important to realize that PML is mostly diagnosed because of the occurrence of clinical deficits, hence representing an advanced stage of disease with diminished treatment success. Moreover, recovery of JCPyV-specific immunity may not be achievable in sufficient time following reduced immunosuppression. Recent case reports suggest that administration of cytokines without or with PML-specific JCPyV VP1 mutant might be worth testing in clinical trials [173, 174]. Unfortunately, an early marker of PML risk is presently lacking that could guide an early intervention similar to BKPyV viremia in PyVAN after kidney transplantation.

35.6 KIPyV and WUPyV

The role of KIPyV and WUPyV in transplant patients is only emerging. In retrospective PCR study of respiratory secretions from 200 immunocompromised patients from France, KIPyV and WUPyV were detected in 8 % and 1 %, respectively [175]. KIPyV was significantly more frequent (18 %) among 45 allogeneic HCT patients tested. In several of these patients, stool samples were found positive for KIPyV [175]. Data from a large prospective study of more than 200 allogeneic HCT patients reported detection rates of 17 % for KIPyV in respiratory secretions, yet without seasonal variation and without prominent respiratory symptoms, but frequent detection of other potential pathogens [176]. Thus, the data suggest that WUPyV and KIPyV may play a role in mostly non-severe respiratory tract pathologies. Clearly, the association with respiratory and gastrointestinal disease requires further study [177]. A significant step in this direction may come from novel antibodies used for immunohistochemistry of lung pathologies, that would be instrumental in better defining KIPyV and WUPyV disease [178, 179].

35.7 MCPyV

35.7.1 Merkel Cell Carcinoma (MCC)

MCC is a rare, cutaneous malignancy first described in 1972, showing the neuroendocrine characteristics of the epidermal Merkel cells that transmit the sensation of touch. The incidence of MCC is increased in immunocompromised populations including those with hematologic malignancies such as chronic lymphatic leukemia, HIV-AIDS, and SOT. Transplant Tumor registries have reported an incidence of MCC in the last two decades with estimated incidence rates of 0.1–0.9 % [180]. Recent data from Finland identified three cases in 4200 renal transplants, all of whom died within 0.5–2.1 years [181]. The risk of MCC increases with duration and intensity of immu-

nosuppression posttransplant. The mean age at diagnosis in SOT patients is 47 years. MCC is aggressively metastasizing in lymph nodes, lungs, liver, and bone, with a mortality rate of 56 % and accounts for 4 % of all skin cancer fatalities after SOT [182]. Treatment is not validated, but should be aggressive with local surgery and significant reduction or discontinuation of immunosuppression, which may lead to regression of MCC metastasis in line with immunologic sensitization to tumor antigens including possibly MCPyV LTag truncations [182–184].

MCC has a predilection for the dermis in sun-exposed areas, and local metastasis is rapid and determines outcome. MCC presents as three types (trabecular, intermediate, small cell, mixed) of which the intermediate type showing large tumor nests is most prominent and frequently diagnosed, while the trabecular type of bridged tumor cell strands or the loosely collagen infiltrating small cell type a rare. Mixed differentiation involving squamous, glandular, and melanocytic suggests a likely common tumor stem cell origin. Moreover, the presence of MCC within other malignancies such as squamous cell cancer and basal cell cancer has been described and complicates the diagnosis [182]. Immunohistochemistry for cytokeratin 20, neuron-specific enolase, and epithelial membrane antigen is positive, while S100, CD45, and laminin staining is frequently negative.

The pathogenetic role of MCPyV support the association with Merkel cell carcinoma, but not with other non-melanoma skin cancer types or prostate carcinoma [185–187]. In the original description, MCPyV-DNA could be amplified in eight of ten MCC tumors by using MCPyV-specific PCR and Southern hybridization. In a control group, only 5 of 59 samples were positive, indicating a positive association between MCPyV-DNA and MCC [188]. In another recent report, MCPyV was detected by PCR in 54 % of 13 MCC, but in only one case of 37 keratoacanthomas, and not in any of 85 squamous cell carcinoma, 28 Bowen's disease, and 6 actinic keratosis [185]. Thus, the association of integrated MCPyV is strong, but not all exclusive. The identification of truncated LTag fusion transcripts as well as small and middle Tag in MCC is in line with activated host cell proliferation, inactivation of apoptosis and genomic instability as key events transforming an infected Merkel cell precursor [182–184]. The predilection of sun-exposed areas in chronically immunosuppressed individuals suggests additional DNA damage and local immunodeficiency. The need for cofactors is also supported by the high seroprevalence and the detection of MCPyV in the general and at risk population [189].

35.7.2 Other PyV-Associated Pathologies

Relevant PyV pathologies include BKPyV-associated ureteric stenosis [190, 191], BKPyV-associated pneumonitis [192], BKPyV-associated CNS disease, some presenting as meningoencephalitis, as systemic disease, or PML [58, 193–198]. More recently, detection of WUPyV has been reported in an HIV-1 infected patient with clinical and radiological signs of PML [199]. Oncogenic transformation by HPyVs may result from uncoupling of EVGR-driven host cell activation from LVGR expression leading typically to host cell lysis and release of viral progeny [200, 201]. As discussed previously, corresponding genetic accidents involving the noncoding control region and/or chromosomal integration are more likely in chronically immunosuppressed individuals exposed to persisting high-level PyV replication in a genetically unstable host cell [127, 129]. These genetic alterations of virus and host cell may then lead to BKPyV carcinoma [202–205]; JCPyV-associated colon cancer after liver transplantation [206]; or after PyVAN in kidney transplants [50]. In the recent years, a number of other intriguing case reports have implicated several new HPyVs in human diseases, with a predilection for the skin (TSPyV, HPyV7, NJHPyV13) [38, 207, 208], or the gastrointestinal tract (HPyV10, MXPyV, MWPyV, STLHPyV11) [37], as summarized in Table 35-1. In the years to come, a more detailed understanding of these HPyVs and their potential pathologic role in transplantation can be expected [13].

35.8 Human Papillomavirus (HPV) Infection

The infectious nature of warts was described in the nineteenth century, followed by demonstrating a viral etiology through the transmission by cell-free extracts in the early twentieth century (Latin: *papilla*, "nipple"; Greek: *-oma*, for tumor). Members of the *papillomaviridae* family are widespread among higher vertebrates, but are considered to be species-specific. Today, more than 100 HPV genotypes can be distinguished by sequencing the major HPV capsid protein L1 [8, 10]. Clinical concepts largely use the distinction between cutaneous, mucous, and anogenital HPV illustrated by their most frequent representatives representatives (Figure 35-3).

HPV virions are non-enveloped icosahedral capsids of 45–55 nm diameter and therefore slightly larger than PyV particles, but are similarly resistant to environmental inactivation. The double-stranded viral DNA genome has approximately 7900 bp. The upstream regulatory region (URR) drives the expression of the early viral E genes (E1, E2, E4, E5, E6, E7), followed by expression of the late viral L or capsid genes (L1, L2) (Figure 35-4). The E3 does not code for a protein, and some E genes are missing from certain HPV types. Overall, HPV E proteins coordinate viral gene expression, maintenance and replication within the host cell and delay host cell differentiation (acanthosis, koilocytosis, parakeratosis, hyperkeratosis). E1 and E2 participate in replication and maintenance of the episomal HPV genome. E5, E6, and E7 have the potential to promote malignant transformation through a variety of activities including inhibition of apoptosis, p53 inactivation and degradation, inhibition of

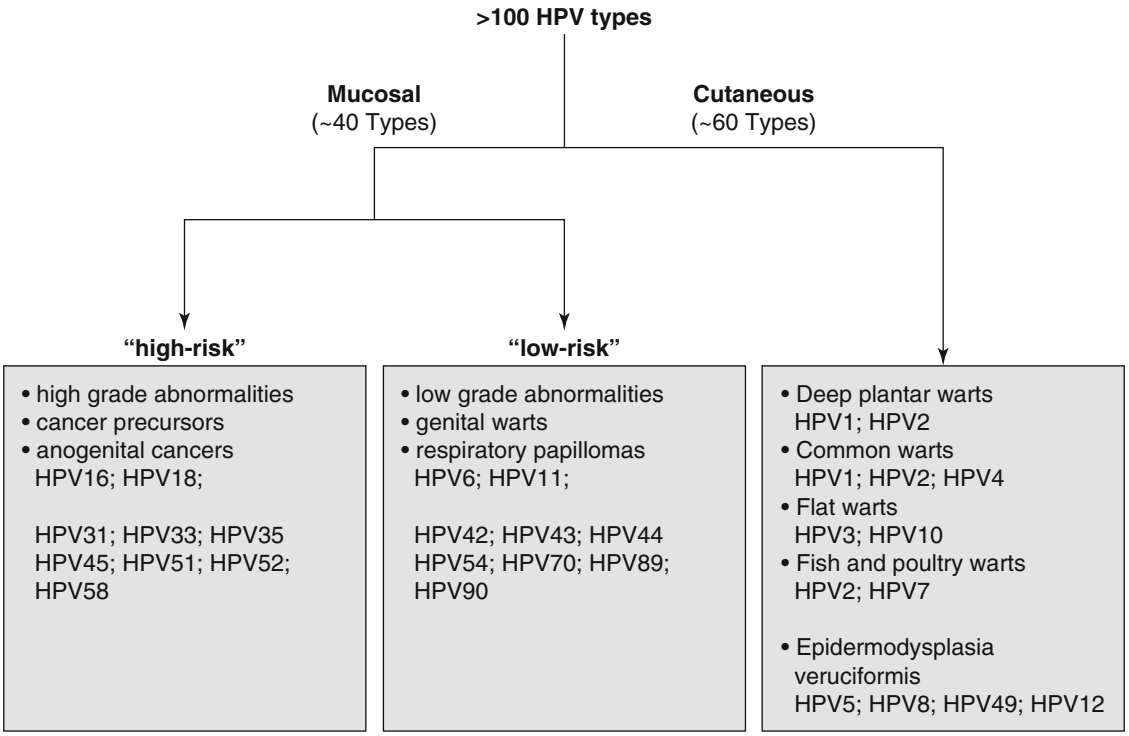

FIGURE 35-3. HPV types and tropism.

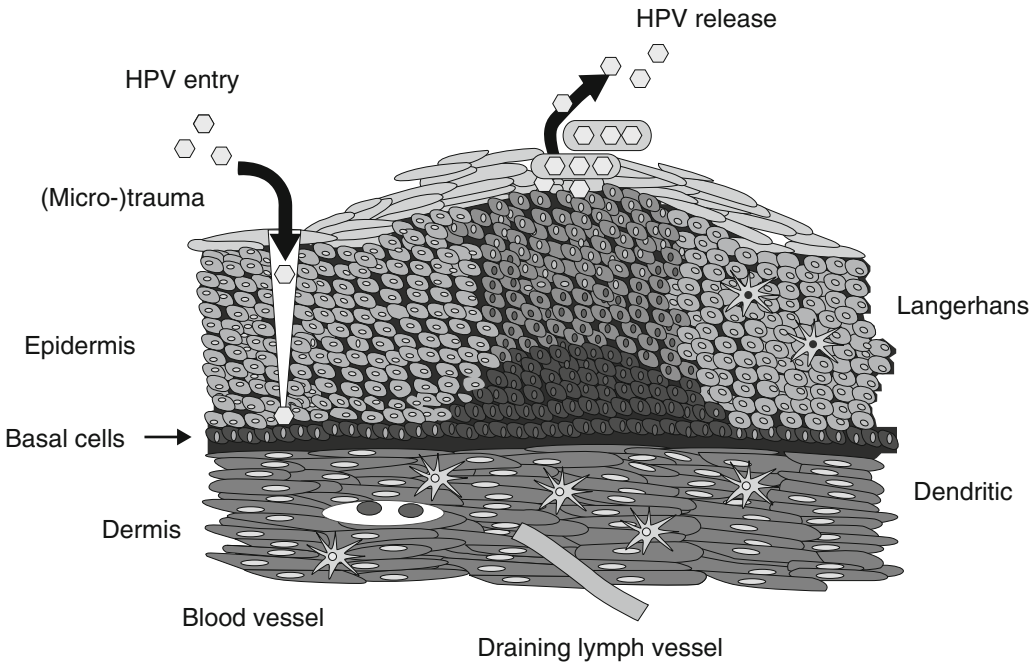

FIGURE 35-4. HPV infection and pathology in the skin. (Micro-)trauma facilitates access of HPV to basal cell layer. Expression of early E genes (*red*) increase proliferation rate to expand the infected cell pool and delay differentiation-driven late L gene (*green*) expression and virion assembly in keratinocytes.

keratinocyte differentiation, and interfering with pRb 107 and 130 inhibition of the E2F transcription factor required in the S-phase of the cell cycle [209, 210]. Deregulated expression of E6 and E7 is linked to malignant transformation and frequently follows the integration of the HPV DNA genome [209, 210]. The HPV virion mainly consists of two structural proteins called L1 of 54 kDa being the major capsid protein sufficient to form virus-like particles bearing the type-specific neutralizing epitopes, and L2 of 63 kDa that binds to the viral DNA and facilitates entry into the nucleus.

HPV infections occur worldwide with little geographic variation. Most data are available for cervical variants detected in women. HPV16 and HPV18 are the most common types in the Western world, whereas HPV52 seems to be more common in Eastern Asia. Cutaneous HPV infections can be detected in healthy skin in more than 80 % of the general population. In a US survey of >4000 persons, the seroprevalences of HPV types 6, 11, 16, and 18 among female subjects were 17.0 %, 7.1 %, 15.6 %, and 6.5 %, respectively [211]. Among males, the seroprevalences were lower for each type, i.e., 6.3 %, 2.0 %, 5.1 %, and 1.5 % for HPV6, 11, 16, and 18, respectively [211]. Comprehensive studies covering 37 HPV serotypes in an Australian cohort of 390 individuals and 34 HPV serotypes in 441 European SOT patients identified seropositive response to at least one HPV in more than 80 %, a dominance of cutaneous beta-HPV types, and demonstrated stability of the antibody response over 48 and 18 months, respectively [212, 213]. A systematic review of HPV infection in heterosexual men reported detection ranges of 1.3–72.9 % with more than half of the studies' rates >20 % [214]. For male college students, higher rates than those reported for females were found [215]. Risk factors for detectable HPV were younger age at first sexual contact, greater number of lifetime and recent sexual partners, higher frequency of sexual intercourse, consistent condom nonuse. Mucosal HPV types in women are most commonly found with sexual activity with an average of 25 %, with similar risk factors as outlined above. Typically, multiple HPV coexist in about 25 % of women including the high-risk types HPV16 and HPV18. Similar data exist for men, but generally smaller study populations. Cervical infections are frequently cleared within 6 months, but persistence is a risk factor for progression to higher grade cervical intraepithelial neoplasia (CIN) and cervical carcinoma. The HPV detection rate increases in immunocompromised patients including HIV, cancer, chemotherapy, and particularly transplantation [216, 217]. In a long-term study from the Netherlands, beta-HPV seropositivity at transplantation in a population of mostly kidney and kidney–pancreas recipients significantly predicted the development of keratinocyte cancer and basal cell carcinoma after 22 years with hazard ratios of 2.9 (95 % CI 1.3–6.4) and 3.1 (95 % CI 1.2–8.0), respectively, and borderline trends for squamous cell carcinoma (hazard ratio 2.9, 95 % CI 0.99–8.5) [218]. The increased risk for HPV-related malignancies may actually start during the period of the underlying disease, at least for renal transplantation, as suggested by an observational cohort study from Denmark. Comparing 12,293 patients with end-stage renal disease patients with 229,524 controls, the age-, gender-, and comorbidity-adjusted risk was 2.41-fold higher (95 % CI 1.83–3.16) for any HPV-related cancer [219].

In immunocompetent individuals, the frequency of clinical HPV manifestations is much lower than the molecular detection rates of the viruses. This indicates that cofactors are required for the clinical manifestations which include failure of specific immune responses, host genetic factors, (micro-) trauma, sunlight, and other injuries (Figure 35-4). In transplant patients, a special role has been attributed to calcineurin inhibitors leading to impaired p53-mediated DNA damage responses, which together with sun exposure promotes genetic alterations in inflamed skin with activated regenerative responses [210, 217]. Benign common warts affect up to 20 % of school children, which then regress, often spontaneously. Risk factors for plantar warts are heated swimming pools and lack of protective footwear. Flat warts are found in up to 10 % among 10-year-olds, whereas plantar warts affect mostly adolescents. Recurrent respiratory papillomatosis in its juvenile form is linked to vaginal delivery and low-risk HPV6 and HPV11, especially in young mothers with condylomata acuminata. The adult form has been linked to a higher number of sexual partners and oral sex. Examples for host genetic factors are mutations in the loci EV1 and EV2 on chromosome 17 and 2, respectively, which encode for transmembrane channel proteins EVER1/TMC6 and EVER2/TMC8 in the endoplasmic reticulum for the precancerous epidermodysplasia verruciformis [220]. Progression to cancer is observed in 30–50 % of affected individuals after 40 years of age [221]. Anogenital warts are frequently caused by HPV6 and HPV11. In men, the preputial cavity or the penile shaft is more frequently affected when uncircumcised or circumcised, respectively. In women, the vaginal introitus is mostly affected and less frequently the vulva. Cervical intraepithelial neoplasia (CIN) and its progression to cervical cancer is linked to HPV16 and HPV18 and has defined cytological (Papanicolaou grades I–V) and histological changes (CIN1, mild dysplasia, CIN2, moderate dysplasia; CIN3, severe dysplasia and carcinoma in situ; invasive cancer across the basal membrane). CIN1 undergoes regression in 90 % of cases, whereas persistence of CIN2 and CIN3 is associated with progression to invasive cervical cancer 60–70 % of cases [222].

35.9 HPV Disease in Transplant Patients

35.9.1 Warts, Precancerous Skin Lesions, and Non-melanoma Skin Cancers

Cutaneous warts and precancerous kin lesions are quite common in SOT patients and increase form 15 % at the time of transplantation to >80 % after 20 years. Sun-exposed areas in the face, neck, forearms, and hands are most commonly seen,

and HPV1 and 2 are most frequently detected [216, 220, 223]. Actinic keratosis in sun-exposed areas, mostly in the face, is associated with HPV5 and HPV8, and affect up to 40 % by 5 years post-SOT may be clinically indistinguishable from precancerous changes or squamous cell carcinoma [220]. Improved detection combining PCR and histological assessment revealed a high rate of active beta-HPV replication in the skin of kidney transplant patients [224] suggesting that surveillance and treatment strategies can be improved. Despite local treatment with liquid nitrogen, electrocoagulation, CO_2 laser, and interferon-alpha, recurrences are frequent. Surgery is commonly avoided for early stages because the role of skin lesions for infection and spread of HPV. An interesting case of presumably donor-derived HPV-positive plane lesions on the hand and forearm of a double hand transplant recipient was reported recently, which increased with increasing tacrolimus concentrations and responded to topical cidofovir treatment [225]. Similarly, rapid progression of flat warts and genital wart have been described in cases after allogeneic HCT in keeping with the contributory role of the allogeneic constellation between host cell and immune effectors [226, 227].

Skin malignancy was documented in 187 cases (19.2 %; mean age 56 years) of a cohort of 976 kidney transplant patients from England amounting to 141 events per 1000 patient years with a mean time to diagnosis of 8 years post-transplant [228]. In two-thirds, skin cancer was in highly sun-exposed areas, with multiple lesions in more than half of the patients. Squamous cell carcinoma was the first and most frequent type in more than half of the affected patients (71.4 /1000 patients at risk), followed by Bowen's disease (23.1 %; 32.5/1000); basal cell carcinoma (15.9 %; 22.4/1000), keratinic actinosis (6.6 %; 9.26/1000); malignant melanoma (0.4 %; 0.53/1000). Risk factors identified were increasing age at transplantation, total time of exposure to immunosuppression, increased creatinine levels at 1 year, and deceased donor graft. Higher rates have been observed in Spain and Australia [220, 221, 228]. In other studies, an association with certain HLA-type has been reported, whereby HLA-A11 decrease, and HLA-B27 and -DR7 increase HPV susceptibility [220, 221]. The HPV types associated with squamous cell cancer are mostly HPV2, HPV5, HPV8, and, interestingly, even HPV16 and HPV18, which are not found in the epidermis of immunocompetent individuals. Lymph node metastasis can occur in about 10 % patients providing an important argument for regular clinical screening and early treatment of precancerous lesions to avoid late presentation (Figure 35-5). Treatment is directed at the physical local destruction of lesions by keratinolytic therapies (salicylic acid), chemical (podophyllotoxin, trichloroacetic acid) or physical means (cryotherapy, laser, surgery). Through this local tissue destruction, opportunities for new HPV infections may be generated, but at the same time, activation of professional antigen-presenting cells (Langerhans, dendritic cells) may actually be the first step of immune activation. Local imiquimod, a toll-like receptor activator and local interferon-alpha therapy may also act along this rationale. Local cidofovir cream may be an adjunct therapy. Therefore, as HPV pathology is largely governed by exposure to immunosuppression, reducing immunosuppression to the lower limits should be considered, or, in case of life-threatening progression of infections, immunosuppression should be discontinued.

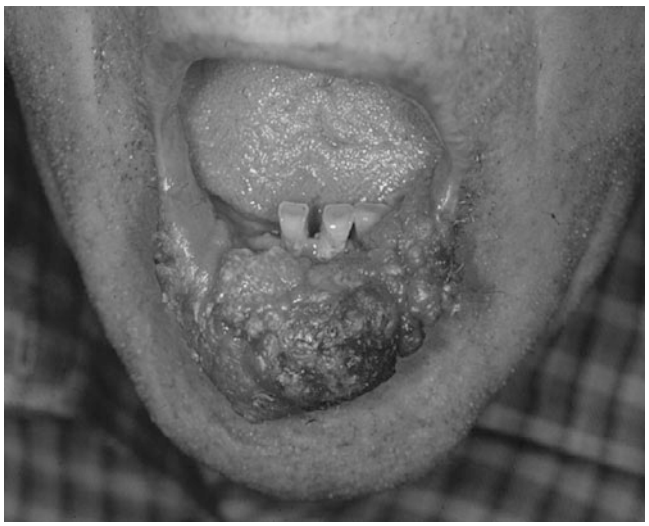

FIGURE 35-5. Squamous cell carcinoma due to HPV in a kidney transplant recipient (courtesy of Peter Itin, MD, University Hospital Basel, Switzerland).

35.9.2 Mucosal Epithelial Cancer

The incidence of invasive cervical cancer also increased after transplantation by three- to fivefold. In kidney transplant patients, however, an excess of cervical cancer incidence was already noted during renal replacement therapy prior to transplantation, as compared to patients before renal replacement therapy [219, 229]. In a large US-based study, the standardized incidence ratios in SOT patients were compared to the general population and a 3.3- to 20.3-fold increased risk of in situ HPV malignancies was identified. This rate increased further with time posttransplant, and implicated the HLA-B44 for susceptibility, whereas the HLA-DRB1:13 was linked to resistance [230]. Higher tacrolimus levels were associated with a higher risk of oropharyngeal cancers, but a lower risk of anogenital cancers, whereas no association with cervical cancers was found. After allogeneic HCT, however, an increased risk of cervical dysplasia has been found in women surviving more than 3 years after allogeneic HCT. Two-thirds of women with normal gynecological findings prior to transplant developed cervical abnormalities, and 25–33 % developed high-grade intraepithelial lesions posttransplant. Importantly, vulvovaginal chronic GVHD was consistently the most significant risk factor in several studies [231, 232]. Although the efficacy of vaccination after allogeneic HCT is known to be limited, HPV vaccination is recommended at 6 and 12 months posttransplant for girls aged 12–17 years of age, and

may be extended to female and male adults [233]. In SOT, suboptimal immunogenicity of the quadrivalent HPV6, 11, 16, and 18 vaccine was observed, when given early posttransplant to lung transplant patients and those with higher tacrolimus levels [234].

In deterministic Markov models, costs and health outcomes of cytology screening and HPV vaccination after kidney transplantation were investigated. Annual screening for cervical cancer by Papanicolaou cytology was considered cost-effective. The role of HPV vaccination in transplantation could not be well evaluated due to lack of data on vaccine efficacy in this setting [222, 235]. After allogeneic HCT, patients requiring continued immunosuppressive therapy for chronic graft-versus-host disease had the highest risk of HPV-related malignancies (odds ratio 4.6 $P=0.019$) underscoring the importance of regular gynecologic assessment before and after transplantation for early treatment [236, 237]. More extensive lesions require surgical resection (conization) and for invasive cancer appropriate staging and chemotherapy together with improved immune functions. The role of therapeutic vaccines in early and late stage is an area of considerable research [222]. Patients after allogeneic HCT are also at risk for oral cavity malignancies. In a systematic review, 64 cases of oral malignancy were identified which were diagnosed 5–9 years posttransplant and primarily affected the tongue, the salivary gland, the lip, and the buccal musosa [238]. Although in most cases, specifics of HCT were lacking, total body irradiation and chronic graft-versus-host disease implying corresponding immunosuppressive treatment appeared as notable risk factors. Since chronic graft-versus-host-disease affects approximately 40 % of allogeneic HCT patients and in practically all cases the oral mucosa, precancerous and cancerous changes may be difficult to diagnose [239] and a classification for HCT patients has been recently proposed (Grade 0: No involvement; Grade I: erythema and/or hyposalivation; Grade 2: lichenoid changes; Grade 3: ulceration, tumor) [238]. Given the potential role of HPV in this setting, systematic studies are clearly needed to evaluate their contribution.

35.10 Conclusion

HPV and PyV are long established viral companions of human populations, but represent a significantly emerging clinical problem in a world of rapidly changing epidemiology among immunocompetent individuals. The increasing number of severely immunocompromised patients, particularly those after successful SOT and HCT, exacerbates their pathogenic potential. Awareness of the pathogenesis, risk factors, and diagnostic tests may hopefully enable the development and better use of appropriate preventive and therapeutic measures.

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36
Hepatobiliary Infections After Solid Organ or Hematopoietic Cell Transplantation

Anne M. Larson and George B. McDonald

36.1 Introduction

The frequency as well as severity of hepatobiliary infections has declined in transplant recipients, due to molecular diagnostic methods, antimicrobial prophylaxis, and preemptive therapies for viral infections [1, 2]. Current drugs in development aimed at viral liver infection have the potential to make these infections rare events after transplantation [3–5].

36.2 Hepatobiliary Infections After Solid Organ Transplantation

Lifelong immune suppression places SOT recipients at risk of infection due to bacterial, viral, and fungal pathogens [1, 6, 7]. Most SOT-related hepatic and gastrointestinal issues relate to graft dysfunction, adverse effects of medications, infections, or malignancy [8–10]. Infectious complications cause significant morbidity and mortality in the 6 months following SOT [11, 12]. Up to 16% of SOT recipients will develop infections after 6 months posttransplant [12, 13]. Anti-infective prophylaxis, more intense surveillance, and preemptive treatment reduces the frequency of these infections [14]. If untreatable life-threatening infection should develop after kidney transplant, immunosuppressive drugs can be discontinued and the patient maintained on dialysis. This option is unavailable to recipients of other organs. Liver infections can develop following any SOT, either isolated to the liver or as part of a systemic infectious process. The transplanted liver presents unique infectious complications related to the liver transplant surgery itself [15].

36.2.1 Viral Infections

Viral hepatitis B (HBV) or C (HCV) infection that is present prior to orthotopic liver transplantation (OLT) can reinfect the new graft with varying long-term consequences. Opportunistic viral infections are more likely to develop after the first month, with herpesvirus infections predominating [11].

36.2.1.1 Hepatitis Viruses A, B, C, D, E

The most common causes of liver injury after SOT are chronic HBV or HCV. Chronic HEV infection, however, is now being identified as a source of posttransplant liver injury and chronic hepatitis (4–6% in Europe) [16, 17]. The prevalence of HCV or HBV infection ranges from 5% to 66% of kidney transplant (KT) and kidney-pancreas transplant (KPT) recipients, depending on country of origin [18]. The effect of HCV on patient and graft outcomes remains somewhat controversial [19, 20], with studies showing inferior outcomes in KT patients who are HCV or HBV-infected [21–24]. A meta-analysis showed that the presence of anti-HCV antibody carried a relative risk of 1.79–1.85 for death and 1.56 for graft failure [23, 25, 26]. HCV-infected patients are at greater risk for developing chronic rejection of the kidney, proteinuria, other infections, glomerulonephritis, and new-onset diabetes, all of which contribute to inferior patient and graft survival [18]. Cirrhotic patients who undergo KT have a significantly worse 10-year survival than those without cirrhosis.

HCV antiviral therapy with interferon-alpha and ribavirin was not possible after KT due to increased risk of allograft rejection, but improved outcomes are expected with direct-acting antiviral drugs for HCV infection. HBV antiviral therapy has significantly improved clinical outcomes following KT and KPT [27, 28]. Antiviral medications should be considered in HBsAg-positive KT patients. Without this protection, acute HBV exacerbation frequently occurs and is often severe [29]. The duration of HBV antiviral prophylaxis following renal transplantation remains unclear [28].

Recurrence of HCV and HBV infections following OLT is common [30–33]. HCV replication in the liver allograft begins within the first postoperative week [34], followed by

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ALT elevation within the first 6 months, then by accelerated fibrosis progression with decreased 5- and 10-year survival compared to OLT recipients without HCV [35, 36]. Approximately 2–6% of HCV-infected recipients develop a rapidly fatal form of recurrence—fibrosing cholestatic hepatitis C [37, 38]; up to 75% develop signs of liver damage; and 30–40% will progress to cirrhosis within 5 years with accelerated graft loss [35, 36, 39–42]. Risk factors for worse outcomes from recurrent HCV include donors >50 years, coinfection with CMV or HIV, and possibly other factors (donor steatosis, presence of ischemia–reperfusion injury or cold ischemia time) [34]. Eradication of HCV would be ideal, preferably before OLT, to prevent infection in the allograft. Interferon alfa-based therapies were poorly tolerated by patients with end-stage liver disease—only 40% of OLT candidates were able to receive therapy and the cure rates were 20–30% [34, 43]. In 2011, the protease inhibitors telaprevir and boceprevir plus interferon–ribavirin were found to improve the cure rate in advanced HCV infection to 50–60% overall; many cirrhotic patients did not tolerate this therapy [34]. Boceprevir and telaprevir increased the $AUC_{cyclosporine}$ by 2.7-fold and $AUC_{tacrolimus}$ by 17-fold, necessitating careful management of immunosuppression [43, 44]. Newer DAAs have now been developed, with cure rates over 90%, and these will revolutionize the treatment of HCV infection in the OLT setting [4, 45–50]. At the time of this writing, the field is rapidly evolving and multiple DAAs are being FDA- and EMEA-approved. We recommend this website for continuously updated information: http://www.hcvguidelines.org.

In the past, patients transplanted for chronic HBV infection developed rapid reinfection of the liver graft, with significant morbidity from fibrosing cholestatic hepatitis B, even in the setting of grafts from HBcAb-positive donors [51]. If antiviral prophylaxis is not given, de novo hepatitis B develops in as many as 58% of recipients receiving an HBcAb-positive graft [51]. HBV recurrence is prevented in >90% of OLT recipients with Hepatitis B immune globulin (HBIG) and antiviral medications. Prophylactic antiviral therapy needs to be continued indefinitely; oral antiviral medications with a high barrier to resistance are used (entecavir, tenofovir) [52, 53].

Although acute hepatitis E virus (HEV) infection is generally self-limited in immunocompromised patients, chronic HEV infection can lead to significant liver injury, advanced fibrosis and cirrhosis [54, 55]. Graft loss after OLT has been reported. Ribavirin monotherapy (600 mg daily) for 3 months is effective for chronic HEV infection [56, 57], and responses have been seen with acute HEV infection [58]. Hepatitis E virus infection has been identified following LT in about 2% of recipients [57].

There are limited data regarding outcomes of HCV- and HBV-infected patients in the setting of heart transplant (HT), lung transplant (LT), and heart–lung transplant (HLT). Reports are conflicting, with some suggesting no difference in mortality, significantly increased all-cause mortality, or increased liver related deaths after HT in HCV-infected patients [59–64]. Data are even more limited in the setting of LT and HLT. Chronic HCV infection remains a contraindication to LT in most centers, although 2–3% of patients transplanted are listed as being seropositive for HCV [60, 65]. Newer DAAs will change the approach to these patients, but have not been tested in these patients as of this writing. The presence of HBV following HT, LT, and HLT does not appear to affect posttransplant survival to 5 years [64, 66]. Antiviral management is the same as that for other recipients with HBV infection.

36.2.1.2 Herpesviruses—HSV, VZV, CMV, EBV, HHV

Cytomegalovirus (CMV)

The seroprevalence of cytomegalovirus in US adults is about 50% (range 30–97%) [67, 68]. CMV is the dominant viral pathogen occurring within the first year after SOT [69–71]. CMV infection is defined as the presence of viral replication (detection of viral proteins or virus in any body fluid or tissue specimen), while CMV disease is defined as infection accompanied by clinical signs and symptoms [72–74]. CMV disease is further classified as CMV syndrome (fever, malaise, neutropenia or thrombocytopenia, and detection of CMV in the bloodstream) or tissue-invasive CMV disease.

CMV tends to invade the grafted organs and thus, presents differently with each organ type [75]. Several factors predispose to CMV infection [69, 70]: (1) Increased immunosuppression (e.g., antilymphocyte antibody or high-dose mycophenolate mofetil therapy) [11]; (2) CMV-negative recipients who receive a CMV-positive graft [69, 70, 76]; and (3) graft rejection with intensified immunosuppression or coinfection with immunomodulating viruses (i.e., HHV-6, HHV-7), bacteria or fungi [11, 77, 78]. In the absence of prophylaxis, symptomatic CMV disease occurs in up to 60% of SOT recipients, with a peak incidence 4–6 months after transplantation—once antiviral prophylaxis has been discontinued [67, 79]. About 80% of CMV disease in SOT recipients is gastrointestinal [80].

CMV disease prevention is with either antiviral prophylaxis (ganciclovir or valganciclovir) or preemptive therapy (treating if CMV viremia develops) [70, 81]. CMV antiviral prophylaxis delays the onset of infection—many recipients will develop infection after prophylaxis is discontinued [12]. CMV drug resistance has been reported with both strategies. CMV disease is generally treated with valganciclovir, ganciclovir, foscarnet, or cidofovir [73, 82].

Renal recipients are at less risk for CMV than other SOT recipients, with an incidence of infection between 8% and 32% [83–86]. Gastrointestinal and liver CMV disease can be seen. The development of CMV replication is associated with renal graft rejection with a hazard ratio of 1.58

(95% CI, 1.16–2.16, $p=0.02$) [87, 88]. In high-risk candidates, antiviral prophylaxis for 3–6 months is preferred to the preemptive approach [73].

The risk of CMV infection or disease following OLT depends on the serologic status of the donor/recipient pair—highest in a seronegative recipient receiving a seropositive donor (D+/R-) [69, 89]. Recipients can also develop reactivation of endogenous CMV, become superinfected by the donor's CMV (D+/R+), or develop CMV from another source [90]. In high risk OLT recipients, level I evidence supports the use of antiviral prophylaxis for 3–6 months [73]. After OLT, hepatitis is the most common manifestation of CMV, generally occurs beyond the first month, and can be more severe than in recipients of other organs [91–94]. Chronic graft dysfunction is an independent risk factor for late CMV disease (HR 6.5, 95% CI 1.7–24.6) [13]. CMV-infected OLT patients are seldom jaundiced but often have elevations in serum aminotransferases (usually <5-times normal), which can be confused with rejection. The diagnosis can usually be confirmed by detection of CMV DNA in the bloodstream but a liver biopsy may be needed. The histologic picture is of an acute hepatitis, with lobular microabscesses (neutrophils), microgranulomas, and rarely cells containing intranuclear or cytoplasmic inclusions, and positive IHC. Asymptomatic low-level CMV viremia usually does not require antiviral therapy but the exact threshold that would trigger therapy is not known [95]. CMV has been associated with an increased risk of acute or chronic graft rejection [87, 96]. The proposed mechanism is that CMV causes hepatic inflammation leading to T-cell activation, altering the state of graft tolerance and triggering rejection [97, 98]. The hazard ratio for development of acute graft rejection within 4 weeks of detection of CMV replication is 2.21 (95% CI, 1.54–3.17, $p<0.001$) in OLT recipients [87]. Residual viral DNA persists in the hepatocyte and biliary epithelium following antiviral therapy, contributing to chronic allograft rejection [99]. CMV is also a risk factor for invasive fungal and bacterial infections, leads to activation of HHV-6 and EBV, and may accelerate hepatitis C virus pathogenesis [96, 100, 101].

Recipients of LT and HT can develop CMV infection (15–25%), usually presenting as pneumonitis, but hepatic CMV infection remains a cause of morbidity [76]. Within 4 weeks of detection of CMV replication, there is an increased risk of graft rejection in HT (hazard ratio 2.6, 95% CI, 1.34–4.94, $p<0.001$) (HR 5.83, 95% CI, 3.12–10.9, $p<0.001$) [87]. Antiviral prophylaxis is recommended for 3–6 months after HT and 6–12 months after LT and HLT.

Intestinal transplant are at highest risk of CMV infections, perhaps because of the amount of lymphoid tissue in the transplanted organ and the intensity of immunosuppression needed [73]. Hepatobiliary disease is not common in this setting, but can occur. Antiviral prophylaxis is indicated for the first 3–6 months following transplantation.

Herpes Simplex Viruses

The Herpes simplex viruses (HSV1 and HSV2) characteristically represent reactivation of latent virus within the recipient. The seroprevalence in the USA for HSV1 is 44–80% and 2–26% for HSV2 [102]; seroprevalence rates are higher in other regions. Manifestations of HSV or VZV infection can develop in ~70% of SOT recipients if antiviral prophylaxis is not used [103]. HSV has tropism for squamous epithelium (nose, mouth, esophagus, genital mucosa), but can involve the intestinal epithelium and liver. Primary HSV infection can be more severe and prolonged in the SOT recipient. HSV reactivation is often asymptomatic; symptomatic lesions may be more invasive or take longer to heal after SOT. Rarely, disseminated HSV can occur, presenting with fever, leucopenia and hepatitis [103, 104]. Severe HSV hepatitis presents with serum ALT levels >5 times normal and may rapidly progress to acute liver failure and death. Histologically, there is significant coagulative necrosis and intranuclear inclusions within the hepatocytes; the diagnosis is readily made by PCR of blood samples and by biopsy PCR or immunohistochemistry. Disseminated disease (including HSV hepatitis) should be treated with intravenous acyclovir. Viral resistance to acyclovir is ranges from 3.6% to 6.3% [104].

The incidence of HSV-related infection in the year following KT/KPT transplant is ~6% [105] and is generally asymptomatic and self-limited, presenting as stomatitis, hepatitis, or pneumonia, and rarely, disseminated disease with severe hepatitis, which is often fatal [106, 107]. Primary infection from the renal graft is rare, especially in an era of acyclovir prophylaxis [107–109]. HSV disease in HT, LT, and HLT recipients presents in similar fashion to KT recipients, with mucocutaneous lesions and only rare instances of aggressive disseminated disease or acute liver failure. HSV1 and HSV2 infections are relatively rare after OLT, but can lead to organ specific disease with hepatic involvement, sometimes culminating in acute liver failure and death; the onset can be very early in the postoperative period (20 ± 12 days) [12, 110, 111]. Given the rapidity of its onset, HSV hepatitis after OLT is most likely a reactivation of the virus; patients present with fever, elevated serum ALT (without jaundice), right upper quadrant pain, leukopenia, and possibly mucocutaneous lesions (31%) [111]. Liver biopsy may need to be performed to exclude acute cellular rejection. Early therapy with intravenous acyclovir significantly improves survival—62% survival in treated patients compared with no survival if untreated [104, 107, 111, 112].

Varicella Zoster Virus (VSV)

VZV may cause hepatitis, with onset between 4 and 12 months after SOT [113]. Primary VZV infection may lead to severe end-organ damage, but few adult SOT recipients are at risk for primary infection because of high seroprevalence

[114]. Reactivated disease generally presents as localized shingles (cutaneous herpes zoster), usually during the first 5 years following transplantation. More rarely, a disseminated, non-dermatomal rash can be seen. Severe, often fatal, disseminated VZV can lead to hepatitis (~50%), pneumonitis (29%), and disseminated intravascular coagulation [114]. LT and HLT recipients have the greatest risk of VZV disease followed by HT, KT, and OLT [115–117]. Prophylaxis with acyclovir, valacyclovir, valganciclovir, or famciclovir reduces early recurrence of VZV following SOT [118]. VZV hepatitis presents with elevated serum ALT (often markedly elevated), fever and abdominal pain in a patient with cutaneous zoster, but an abdominal prodrome without skin lesions can occur. Diagnosis is made by PCR of blood samples or lesions. Histologically, liver biopsies show coagulative necrosis and inflammation [119].

Epstein-Barr Virus (EBV)

EBV infection can be either primary or secondary, with presentations ranging from asymptomatic viremia to infectious mononucleosis and posttransplant lymphoproliferative disorder (PTLD) (see also Chap. 26) [120]. The majority of infections in adults are secondary to viral reactivation and are generally self-limited, resolving with supportive care [121]. Primary infection is often symptomatic and associated with more severe disease, particularly hepatitis (antiviral therapy may be required) [122–124]. EBV infection can also lead to EBV-associated posttransplant lymphoproliferative disorder (PTLD), with an incidence that varies from 0.6% to 16% depending on the organ transplanted and whether the recipient is an adult or a child [125]. PTLD is more likely in those with greater immune suppression [126, 127]. If PTLD involves the liver or the biliary tract, then symptoms of liver injury or obstruction can be seen [128].

Human Herpesviruses 6, 7, and 8

Infection by HHV-6, -7, and -8 are common, but clinical disease is rare after SOT (e.g., <1% of SOT recipients) [129–131]. HHV-6 infects >95% of the general population; variant B is the most common in SOT recipients, with reactivation developing about 2–8 weeks after SOT. The majority of infections are asymptomatic and short-lived infection, but presentations can include fever, rash, bone marrow suppression or tissue invasive disease (hepatitis, encephalitis, pneumonitis, and colitis). HHV-6 may cause a hepatitis-like picture, with elevated ALT, fever, and lymphocytic infiltration and, in liver recipients, graft dysfunction [130, 132–134]. Reports of acute liver failure have been described. Some have shown increased mortality in OLT recipients with HHV-6 reactivation compared to those without [135]. HHV-6 has also been associated with opportunistic fungal infections, earlier and more severe recurrence of HCV, CMV infection, and graft rejection [136]. Diagnosis is by PCR in serum or tissue; serologic testing is of limited use [130, 137]. HHV-6 can be chromosomally integrated, potentially complicating a diagnosis. Prophylactic and antiviral therapy is similar to that in CMV—ganciclovir, foscarnet, or cidofovir, based predominantly on in vitro data [138]. Monitoring for reactivation of HHV-6 and HHV-7 after OLT has not been shown to be beneficial [139].

HHV-8 (Kaposi's sarcoma-associated herpesvirus) is oncogenic [12, 140]. HHV-8 can also lead to Castleman's disease, lymphoproliferative disorders, and primary effusion lymphomas (a form of non-Hodgkin's lymphoma) [141, 142]. Primary HHV-8 infection may also cause fever, bone marrow suppression (leukopenia), and rash, which generally lasts about 10 days [131]. HHV-8 can rarely cause hemophagocytosis, myelosuppression, and multiorgan failure in the SOT recipient [143, 144]. A fourth of Kaposi's sarcoma cases involve the lungs, gastrointestinal tract, and the liver [131].

36.2.1.3 Adenovirus

Adenovirus liver infection after SOT is more common in children than adults, can result from primary infection or reactivation, and may be disseminated or localized [145–148]. OLT patients on high dose immunosuppression are at greatest risk of infection. Adenovirus hepatitis can be severe and acute liver failure may result. PCR for adenovirus DNA in blood, plus a consistent clinical picture, usually suffices for diagnosis. Liver histology shows microabscesses and poorly formed macrophage granulomas in addition to coagulative necrosis [149]. Adenovirus infection after SOT has been successfully treated with cidofovir (the current standard of care) and with ribavirin and intravenous immunoglobulin [145, 150, 151].

36.2.2 Bacterial Infections

36.2.2.1 Common Bacteria

The risk of hepatobiliary bacterial infection (cholangitis or liver abscess) depends on the organ transplanted, with OLT recipients more susceptible because of biliary duct manipulation and potential for biliary tract contamination [152, 153]. The infectious complication rate between deceased donor and living donor OLT is similar at about 67%; however, those receiving a deceased donor liver are more likely to have intra-abdominal infections, usually developing within the first 2–3 months following transplantation [12, 152, 154, 155]. Over time, the pattern of bacterial infection changes [1, 153]. The most common infectious agents in the first 3 months following OLT are gram-negative rods (~50%), gram-positive cocci (44%), and *Candida* species (6%) [156].

Two to six months after OLT, viral infections predominate and opportunistic infections may occur [1, 152]. During this period, less common bacterial infections (*Listeria monocytogenes*, *Norcardia* species, and reactivation of latent infection, including *Mycobacterium* species) can be seen [152]. Six months or more following OLT, bacterial infections are generally related to environmental exposure, late biliary complications, and allograft dysfunction [13, 152].

Risk factors for infection after OLT include preoperative use of antibiotics, surgical-technical issues, steatosis, ischemia–reperfusion injury, and volume of intraoperative blood transfused [12, 152, 157]. The presence of serum endotoxin pretransplant is predictive of risk of bacterial infections post-OLT [158]. The use of antibiotics prior to transplantation in these patients has led to multi-drug resistant organisms, particularly enterococci, *Staphylococcus aureus*, and non-albicans *Candida* species [159, 160]. Hepatobiliary infections after OLT range in frequency from 18% to 37%; are frequently related to complications of the surgical procedure; and surgical site infections are closely related to intraoperative conditions and postoperative events [161]. Surgical site infections after OLT are associated with graft loss and death [152, 153, 162, 163]. The biliary tract is responsible for up to 34% of bacteremias seen following OLT. Biliary leakage and stricture formation, generally at the anastomotic site, are the most common of the biliary abnormalities [164, 165]. Reconstruction of the biliary tree with anastomoses either directly duct-to-duct or via Roux-en-Y can lead to both anastomotic and non-anastomotic strictures, risk factors for cholangitis, especially after Roux-en-Y anastomosis [12, 152, 153, 166]. Both bacteriobilia and fungibilia in OLT recipients undergoing endoscopic retrograde cholangiography for biliary complications are associated with worse survival, even with successful endoscopic treatment [167].

Following OLT, the biliary tree is exclusively dependent upon the hepatic artery for its blood supply. Loss of arterial flow results in bile duct necrosis with leakage and development of intrahepatic bilomas, abscesses, and perihepatic fluid collections. Hepatic artery thrombosis leads to ischemic bile duct injury in 5–8% of OLT recipients presenting with either mildly elevated liver enzymes or bacterial cholangitis or acute liver failure necessitating urgent retransplantation [168–171]. Portal vein thrombosis can also lead to hepatic ischemia and severe hepatic dysfunction if it occurs early in the posttransplant course; later, signs of portal hypertension develop, also a predisposing factor for infection.

Immunosuppression increases the risk of cholelithiasis and cholangitis in non-liver SOT recipients, especially among diabetic patients [172, 173]. The development of cholangitis in this setting carries an increased risk of mortality. Some have advocated for cholecystectomy in these patients regardless of symptoms to minimize further biliary complications in HT and HLT recipients [173, 174]. Bacterial infections following intestinal transplantation are common, often associated with mucosal disruption and bacterial translocation; approximately 17% are from an intraabdominal source and cholangitis has been reported [175].

36.2.2.2 Mycobacteria

The incidence of *M. tuberculosis* infection following SOT (especially KT) is higher than the incidence in the normal population, especially in areas where the prevalence is high [176–178]. Tuberculosis has been reported as early as 2 weeks to as late as 2 years after SOT, usually secondary to reactivation of latent infection. Isoniazid prophylaxis prior to, during and after transplantation can be used with minimal hepatotoxicity [179]. Although the majority of patients develop pulmonary disease (51–73%), disseminated disease, including intra-abdominal infection, is seen in 38% [12, 176, 178, 180, 181]. Hepatic tuberculosis presents with fevers, night sweats, weight loss, and cholestatic liver tests. Non-tuberculous mycobacterial infections (e.g., *Mycobacterium avium* intracellulare, *M. chleonae*, *M. mucogenicum*, *M. triplex*, and *M. xenopi*) are rarer and less likely to affect the hepatobiliary system after SOT [12, 182]. LT recipients are at higher risk of non-tuberculous mycobacterial infection than other SOT patients [183]. Treatment of tuberculosis is complicated by interactions between rifampin and the calcineurin inhibitors (cyclosporine, tacrolimus), whose doses must be increased by two- to fivefold according to blood levels [12, 184]. The use of rifabutin as an alternative to rifampin has shown promise [176].

36.2.3 Fungal Infections

Fungal infections usually develop after the first month after SOT, particularly after discontinuation of fungal prophylaxis, which has decreased the incidence of early fungal infections and subsequent mortality [185]. OLT recipients have the highest incidence (7–42%), followed by pancreas (18–38%), HT and HLT (15–35%), and then KT (0–14%) [186]. Liver involvement is generally secondary to disseminated infection or cholangitis, especially after OLT, where fungal abscesses may develop from *Candidal* species (*C. albicans, C. tropicalis*), with a 12-month cumulative incidence after SOT of 1.9% [187]. Molds such as Aspergillus and zygomycetes are increasing in incidence [100, 187–190].

36.2.3.1 Candida Species

Candidal infections after SOT are most frequently seen in the blood, urinary tract, and in the abdomen; blood cultures do not reliably confirm the presence of invasive disease [191]. Risk factors for invasive candidiasis include prophylactic antibiotics, KT, lengthy operative procedures, ≥40 units of

blood products, prolonged ICU stays, Candida colonization, Roux-en-Y anastomosis, early surgical reexploration, and CMV infection [192, 193]. In the absence of prophylaxis, invasive infections occur in up to 42% of OLT recipients, and *Candida* species account for 60% to >80% [100, 188, 194, 195]. The route by which candidal organisms reach the liver, spleen, and kidneys is via gastrointestinal "persorption," where yeast forms pass through tight junctions into the portal circulation [196]. OLT recipients have significant *Candida* gastrointestinal colonization; antifungal prophylaxis should be given to all adult OLT recipients to prevent invasive disease [197]. About 4% of KT or KPT recipients develop intestinal fungal infections, most often candidal. Pancreas recipients with enteric drainage are at greater risk of fungal infection compared to those with bladder drainage. Small intestine graft recipients develop invasive *Candida* infection at rates as high as 28% [198]. Risk factors include graft rejection, high immunosuppression, anastomotic disruption, abdominal reoperation, and multivisceral transplantation [191]. Prophylaxis is generally administered for at least 4 weeks until the small bowel anastomosis has healed.

Anti-fungal therapy in SOT recipients is similar to that in non-SOT patients [199]. Therapy is generally continued for at least 4 weeks. Invasive lines and catheters should be removed if at all possible when candidemia is present. Azole antifungal medications can have drug–drug interactions with calcineurin inhibitors. Antifungal prophylaxis has reduced the risk of invasive disease and mortality but has led to infections with non-*Candida albicans* species, including *C. glabrata* and *C. krusei* [200–202].

36.2.3.2 Molds and Other Fungi

Invasive aspergillosis (usually *Aspergillus fumigatus*) can develop in SOT recipients, most commonly in LT recipients followed by KT, OLT, and HT recipients, generally in the lungs, secondary to inhalation of spores [203]. Hepatic involvement is not common and isolated hepatic aspergillosis is even rarer, presenting as multiple abscesses within the liver parenchyma, although single abscesses may form [204, 205]. Invasive aspergillosis after KT occurs later than after OLT, associated with the presence of chronic lung disease and chronic heart failure [206]. After OLT, *Aspergillus* accounts for about 25% of invasive fungal disease, especially following biliary tract infection [200, 207, 208]. Hepatic artery mycotic aneurysms have been described. LT and HLT recipients have the highest incidence of fungal infection in the SOT setting and non-candidal species predominate as a major source of mortality [209–211]. These infections generally involve the transplanted organ and not the hepatobiliary system. Mortality is lower in SOT recipients with invasive Aspergillosis (34.4%) compared to HCT recipients (57.5%) [203]. *Cryptococcus neoformans* is the third most common fungal infection seen following OLT and can infect any SOT recipient, usually presenting as pneumonia (46%) and meningitis (36%) [12]. Hepatobiliary involvement is infrequent, although disseminated disease, including peritonitis, can occur. *Histoplasma capsulatum*, a rare cause of granulomatous liver disease when disseminated, is generally asymptomatic in the immunocompetent host. Clinical infection in SOT recipients is uncommon (<0.5%) [212]. Infection can be primary or due to reactivation, and can progress to disseminated disease with multiorgan involvement and death in ~10% of infected SOT recipients [212–214]. A urinary histoplasma antigen test is the most sensitive diagnostic study. Coccidioides species can also disseminate in immunocompromised hosts [215].

Mucormycosis is more common after HCT than SOT; OLT recipients have an incidence of 0–1.6% [216]. Rarely, mucormycosis can involve the liver, sometimes presenting with Budd–Chiari syndrome [217]. Patients with iron overload syndromes, prior triazole antifungal use, renal failure or diabetes are risk factors; SOT recipients with mucor infections face 49–71% mortality [218].

Pneumocystis jiroveci causes pulmonary disease in SOT and generally develops after prophylactic medications have been discontinued [219]. Hepatobiliary involvement has not been reported.

36.2.4 Differential Diagnosis of Hepatobiliary Problems in SOT Patients

Hepatobiliary problems after SOT can be due to infections or noninfectious problems (especially after OLT), or to a combination. The approach to diagnosis and treatment is based on imaging, molecular diagnostics, and clinical experience ("Man sieht nur, was man weiß. Eigentlich: Man erblickt nur, was man schon weiß und versteht," Johann Wolfgang von Goethe[1]). Table 36-1 displays the major causes of hepatobiliary disease after SOT.

36.3 Hepatobiliary Infections After Hematopoietic Cell Transplantation

36.3.1 Viral Infections

The clinical and histologic manifestations of hepatic viral infection after HCT are not uniform, but range from fulminant hepatic failure (HSV, VZV, adenovirus, HBV) to more indolent, chronic inflammation (HCV, HBV) to a cholestatic

[1] "*You only see what you know. Actually: One sees only what one already knows and understands*."

TABLE 36-1. Hepatobiliary diseases after solid organ transplant, by clinical presentation and by cause [15, 23, 39, 91, 103, 106, 164, 165, 168, 169, 172–174, 217, 220–237]

	Hyperbilirubinemia	Abdominal pain	Elevated liver enzymes (ALT, AP)	Liver failure	Ascites
Infectious causes	HBV, HCV, HEV infection Fibrosing cholestatic hepatitis B or C Herpesvirus, adenovirus hepatitis Bacterial cholangitis Infection-related hyperbilirubinemia (cholangitis lenta)	Cholecystitis Biliary obstruction with cholangitis Fungal, bacterial abscess Acute viral hepatitis Peritonitis after bile leak (OLT)	Viral hepatitis Fungal, bacterial abscess EBV lymphoproliferative disease	Decompensation of cirrhosis Fibrosing cholestatic hepatitis B or C Fulminant viral hepatitis (HSV, VZV, adenovirus)	Budd–Chiari syndrome caused by mold infection Fulminant viral hepatitis
Noninfectious causes	OLT rejection DILI Vascular injury (OLT) Biliary tract obstruction Recurrent hepatocellular carcinoma (OLT) Hemolysis, RBC transfusions, Gilbert syndrome, renal insufficiency	Biliary obstruction (stone, stricture, tumor) Cystic duct obstruction Acute pancreatitis Budd–Chiari syndrome Sinusoidal obstruction syndrome	DILI (azathioprine, sirolimus, CNI) Biliary obstruction Rejection (OLT)	Hypoxic hepatitis Hepatic vascular injury (OLT) Sinusoidal obstruction syndrome Budd–Chiari syndrome	Cirrhosis Fulminant hepatic failure, nonviral Nodular regenerative hyperplasia

HBV hepatitis B virus, *HCV* hepatitis C virus, *HEV* hepatitis E virus, *OLT* orthotopic liver transplant, *DILI* druginduced liver injury, *RBC* red blood cell, *EBV* Epstein-Barr Virus, *CNI* calcineurin inhibitor, *HSV* herpes simplex virus, *VZV* varicella zoster virus.

picture (HCV, as fibrosing cholestatic hepatitis, or CMV biliary disease). Liver biopsy is rarely performed for viral diagnosis, as most hepatic viral infections can be identified by tests for viruses in blood samples. Antiviral treatment is available for almost all common viruses that infect the liver after HCT.

36.3.1.1 Hepatitis Virus Infections Before Transplant

Hepatitis Viruses in Potential Transplant Recipients

Hepatitis C virus. HCV RNA testing might be needed for HCT candidates with negative anti-HCV test results whose history indicates increased risk for HCV infection (e.g., intravenous drug abuse) or whose B cells cannot mount an antibody response [238]. HCV-infected HCT candidates requiring HCT can proceed with HCT with an HCV-infected donor, provided that recipient has full understanding of the risk that long-term consequences of HCV infection after HCT. However, such an approach may be associated with a higher rate of virologic failure (e.g., genotype 3 infection, Q80K NS3 polymorphism) [239]. Data are lacking regarding pre-HCT treatment of HCV-infected HCT candidates [5, 240]. Direct-acting antiviral (DAA) therapy before HCT might be considered for (1) patients with chronic HCV infection; (2) patients with HCV-related cirrhosis and/or portal hypertension; and (3) patients with an HCV-related lymphoproliferative disease [240, 241]. It is not known whether the efficacy of DAA therapy is affected by dysfunctional immunity (following chemotherapy or biologic therapy for cancer) or whether eliminating HCV before HCT improves the outcome of transplant by reducing the risks of post-HCT fatal sinusoidal obstruction syndrome, liver decompensation, fibrosing cholestatic hepatitis, or recurrent lymphoma.

Hepatitis B virus. The major risks faced by HBV-infected patients include fatal sinusoidal obstruction syndrome following some myeloablative regimens if hepatic inflammation or fibrosis is present; liver decompensation if cirrhosis is present at baseline; and fulminant hepatitis B or fibrosing cholestatic hepatitis B (seen only when antiviral prophylaxis has been forgotten or HBV is activated in a patient with occult HBV) [242, 243].

Staging of liver disease in candidates with chronic viral hepatitis. The presence of advanced fibrosis or cirrhosis may have a significant impact on HCT eligibility, risk of hepatocellular carcinoma, and the choice of conditioning regimen [244, 245]. Screening for hepatocellular carcinoma should be carried out before referral for HCT. Detection of advanced fibrosis and cirrhosis may require liver biopsy if transplant decisions would be affected by such findings. Two alternatives to biopsy have not been well-studied in HCT candidates: (1) Analysis of blood test panels (aminotransferases, platelet count, coagulation parameters, gamma-glutamyl transpeptidase, total serum bilirubin, haptoglobin, and gamma globulins) is not recommended in HCT candidates [5]; (2) Vibration-controlled transient elastography may be useful. In patients with chronic HCV infection, elastography has an estimated sensitivity of 70% and specificity of 84% for a diagnosis of significant fibrosis and an estimated sensitivity of 87% and specificity of 91% for a diagnosis of cirrhosis [246]. Elastography data in HCT candidates, particularly those with malignant infiltration or extramedullary hematopoiesis, are limited [247, 248].

Preventing Passage of Hepatitis Viruses from Infected Donors

The risk of viral passage from donors infected by HBV differs from that of HCV-infected donors, as the former can have HBV confined to the liver, whereas HCV-infected donors are always viremic and invariably transmit HCV to uninfected recipients [249]. Prevention of passage of HCV from an HCV-infected donor to the HCT recipient does not require a sustained virologic response in the donor, just the disappearance of HCV from extrahepatic reservoirs (peripheral blood stem cells and marrow cells) [250–253]. When there are oncologic imperatives for moving quickly to transplant, DAAs can clear extrahepatic HCV from donors more quickly than interferon and ribavirin without significant toxic effects on the donor marrow [252]. If time does not permit treatment of an HCV-infected donor, the use of HCV-infected hematopoietic cells for an HCV-uninfected recipient is not absolutely contraindicated because new DAAs can provide a virologic cure in the majority of transplant survivors after recovery and immune reconstitution.

HBV-infected donors who are viremic (serum HBV DNA+) will transmit virus to their recipients [240, 242]. When equally HLA-matched donors are available, an uninfected donor is preferred. If the most suitable donor has chronic hepatitis B, treating that donor with entecavir or tenofovir may prevent passage of virus [254, 255]. HBV persisting in donor peripheral blood stem cells may have to be eliminated to prevent passage [255, 256]. HB_sAg negative, anti-HB_c-positive donors can be used if their serum and peripheral blood stem cells are HBV DNA-negative. HBV-naïve recipients of cells from an HBsAg positive, serum HBV DNA-negative donor should be monitored for HBV DNA after transplant. Hepatitis B vaccination of HBsAg-negative recipients prior to the start of conditioning therapy, using double doses of HBV vaccine, may also be beneficial if there is a risk of passage from an infected donor [257]. When both donor and recipient are infected by HBV, the recipient should be treated with antiviral drug prior to conditioning therapy. Donors with occult hepatitis B (undetectable serum HBV DNA, HBsAg-negative but anti-HBc positive) can also be used; however, in HBV-endemic countries, 6–15% rates of viremia in such patients have been reported [258]. An entity termed "false occult HBV" has been described in Europe in patients with isolated anti-HBc but HBV DNA+ in 2.8% of cases; in some viremic patients, HBsAg mutants, missed by standard multivalent assays, were found [259]. If time permits, both donor and recipient should receive entecavir therapy and be vaccinated prior to conditioning therapy and the recipient should be given entecavir prophylaxis for up to a year after transplant [254, 260]. A donor who is naturally anti-HBc positive may be the preferred donor if the recipient is HBsAg positive or anti-HBc positive, as adoptive transfer of immunity can effect clearance of virus [261]. A similar result may be obtained by immunizing donors before cell harvest and infusion into HBV-infected recipients [261, 262].

Hepatitis D virus, which infects only patients with HBV infection, has not emerged as a significant cause of post-HCT liver disease, perhaps because pharmacologic control of HBV addresses HDV coinfection. Current recommendations are to exclude HDV-infected donors [240]. Recent recognition of the prevalence of hepatitis E virus in both endemic and non-endemic areas has led to awareness that this virus can be transmitted from infected donors and from blood product transfusions to recipients [263, 264]. Treatment of infected donors with ribavirin should be a feasible method of preventing transmission of HEV [58]. Donors infected by hepatitis A virus should not be harvested until they reach a non-viremic state. Screening of donors by serum ALT and anti-HAV IgM tests will detect HAV infection [265].

36.3.1.2 Viral Liver Infections Through the First Year After Transplant

A sudden rise in serum ALT following HCT is usually due to a noninfective cause such as zone 3 hepatocyte necrosis in SOS (peaking around day 20), hypoxic liver injury (as in septic or cardiac shock or respiratory failure), acute biliary obstruction (choledocholithiasis), drug-induced liver injury, or the acute hepatitic presentation of GVHD [242, 266]. If a likely cause is not apparent, acute viral hepatitis should be suspected; HSV, VZV, adenovirus, and HBV can lead to fatal fulminant hepatic failure after HCT whereas hepatic infections caused by CMV and HCV are seldom severe, with the exception of fibrosing cholestatic hepatitis C [242, 267]. With routine use of prophylactic acyclovir/valacyclovir, acute hepatitis due to HSV and VZV is now rare; however, HHV-6 and HHV-8 reactivation and HEV as causes of hepatitis have been reported after HCT [268, 269]. When there is uncertainty about the cause of rising serum ALT levels, serum PCR tests for herpesviruses, adenovirus, HCV, HBV, and, in some circumstances, HEV should be performed [263, 264]. If acyclovir is not being given to a patient with rapidly rising serum ALT, it should be started empirically, particularly if the patient presents with abdominal complaints typical of VZV infection [270].

Adenovirus. Adenovirus hepatitis should be suspected if the patient has concomitant pulmonary, renal, bladder or intestinal symptoms but may present with just fever, raised serum ALT, and hypodense regions in the liver on abdominal CT scan along with features of bone marrow suppression [271]; the most effective treatment is cidofovir when given early in the course of infection but many cases of adenovirus hepatitis are rapidly fatal [272–275]. However, not all patients who are excreting adenovirus and not all viremic patients progress to liver and multiorgan involvement [276]. New agents for prevention and treatment are in development [3, 275].

Herpes simplex virus. Before effective antiviral prophylaxis, HSV activated from latency caused infections far beyond its tropism for squamous epithelium, affecting the lungs, liver, and gastrointestinal mucosa. Hepatic presentation was that of rapidly rising serum ALT, followed by jaundice and liver failure. In a 15-year study of over 6000 patients undergoing HCT during 1992–2007, no cases of severe HSV hepatitis were seen [266].

Varicella zoster virus. In visceral VZV infection, abdominal distention, severe pain, fever, and rising serum ALT levels may precede cutaneous manifestations by up to 10 days [270, 277]. High-dose empiric acyclovir should be started on clinical suspicion while serum is analyzed by PCR for VZV DNA. A common presentation of disseminated VZV is that of enigmatic abdominal pain 2–6 months after HCT, after discontinuation of acyclovir—an indication for intravenous high-dose acyclovir while a definitive diagnosis is sought (VZV DNA in serum). In the first 100 days after HCT, severe VZV hepatitis occurred in 4 of ~2500 patients, all during the 1990s, and none since 1998 [266].

Cytomegalovirus. Liver and biliary CMV infections are now extremely rare after HCT [266, 278]. Even during an era when CMV disease was common, liver CMV infection was almost never a serious medical problem. An autopsy study of 50 patients from the 1980s examined liver tissue by viral culture, immunohistochemistry, and in situ DNA hybridization. Among patients who were seropositive at baseline, viremic during life, with CMV pneumonia and positive CMV cultures of the liver at autopsy, all had CMV-infected liver cells, including hepatocytes, endothelial cells, Kupffer cells, and bile duct epithelium, along with multifocal lobular granulomas [279]. In contrast, CMV infected liver cells were rarely found in the absence of disseminated CMV disease [279]. Patients with CMV in the liver cells did not have a typical clinical picture or laboratory results that were different from HCT patients without liver CMV at autopsy [279]. CMV infection of the gall bladder mucosa and the ampulla of Vater can lead to clinical problems (cholecystitis and biliary obstruction, respectively) [280].

Hepatitis B virus. Fulminant hepatitis and fibrosing cholestatic hepatitis B may develop during immune reconstitution in patients at risk, but can be prevented with prophylactic antiviral agents [281]. Entecavir is currently the drug of choice for this indication because of superior efficacy and fewer mutations [282]. If severe hepatitis from HBV reactivation does occur, usually because a diagnosis of HBV was not made prior to HCT, antiviral therapy with the most potent antiviral drug available (entecavir or tenofovir or both drugs simultaneously [283]) should be initiated immediately; however, progression to fatal liver failure is not uncommon [284]. Fulminant hepatitis B has also been reported following discontinuation of prophylactic antiviral therapy, and all patients, particularly those with high pre-transplant HBV DNA levels, should be monitored following antiviral drug withdrawal [285, 286]. In patients who acquired HBV as infants, the infection may be completely occult during transplant screening (negative serum tests for HBV antibodies, antigen, and DNA); after transplant, viral activation and clinical hepatitis during immune suppression can occur [243].

Hepatitis C virus. Hepatic manifestations include (1) an increased risk of fatal sinusoidal obstruction syndrome among patients with chronic HCV infection who receive sinusoidal endothelial cell toxins [242] as part of conditioning therapy [239]; (2) hepatic inflammation occurring at 3–6 months after HCT, coincident with immune reconstitution and discontinuation of immunosuppressive drugs [239]; (3) liver decompensation among patients who had cirrhosis at the time of transplant [287–289]; and (4) rarely, fatal fibrosing cholestatic hepatitis C in patients receiving mycophenolate mofetil [267]. Extrahepatic manifestations of HCV infection after HCT have been suggested by epidemiologic studies, including an excess of deaths related to bacterial infections and to liver disease [288, 289]. It is not clear whether excess mortality in these reports is due to HCV per se, the presence of undetected hepatic fibrosis and portal hypertension at HCT, or both. Other studies find no increased mortality in follow-up of HCV-infected survivors of HCT, to 10 years [239, 290].

Increases in titers of HCV RNA occur in both cancer patients receiving chemotherapy [240, 291, 292] and in HCV-infected HCT patients, but with the exception of fibrosing cholestatic hepatitis C [267], this viremia is not commonly manifest as a clinical illness. Upon immune reconstitution, there may be increases in serum ALT in ~30% of infected HCT patients [239, 290]. There is little utility in measuring titers of HCV RNA after HCT in patients already known to be infected.

The alternative to pre-HCT therapy for HCV is to treat after HCT, following immune reconstitution if feasible, using DAAs [5]. Sustained virologic response rates of 70–96% have been observed in patients who received DAAs during immunosuppressive therapy after liver transplant, suggesting that similar results would be seen in HCT survivors [46]. DAA therapy after HCT can be deferred, as the natural history of HCV infection in this setting is usually benign, with three exceptions: patients with fibrosing cholestatic hepatitis C [267]; patients with cirrhosis whose condition is deteriorating [287]; and patients who underwent HCT for HCV-related lymphoproliferative disorders [5, 240, 241]. Therapeutics for HCV treatment are rapidly evolving; we recommend this website for continuously updated information: [http://www.hcvguidelines.org]. Monotherapy with a DAA is not recommended regardless

of HCV genotype. Telaprevir-based and boceprevir-based regimens are not recommended for treatment of any patient with HCV infection. HCT recipients are characteristically receiving multiple drugs that have pharmacologic interactions with DAAs or toxic effects that overlap with those of DAAs. Such interactions have not been extensively studied in HCT recipients; what is known is summarized in a recent ASBMT review [5]. Metabolism by CYP450 enzyme, specifically the CYP3A4 isoform, is the major metabolic pathway of approved DAA HCV therapies [293–295]. When a calcineurin inhibitor or sirolimus is used with a protease inhibitor, it is reasonable to empirically reduce the dose of the immunosuppressive agents and monitor their levels more frequently because of major CYP3A4 and P-glycoprotein drug–drug interactions. The field of DAA therapy for HCV infection is rapidly evolving, and attention must be paid to drug–drug interactions in HCT recipients [5].

Hepatitis E virus. HEV may result in chronic infection in immunosuppressed individuals including HCT recipients [17, 269, 296]. In contrast to water-borne HEV due to genotype 1 and 2 found in the developing world, HEV genotype 3 (HEV3) is found in mammalian reservoirs including domestic pigs, wild boar and deer in Europe, North America, and China [268]. Diagnosis is by detection of anti-HEV antibodies and HEV RNA. Oral ribavirin has been shown to be effective therapy [58].

36.3.1.3 Hepatitis Virus Infections in Long-Term Transplant Survivors

Sporadic liver infection by herpesviruses can be seen in long-term survivors whose antiviral prophylaxis has been discontinued, especially those who remain on immune suppressive therapy for chronic GVHD. The more common infections are with hepatitis viruses.

Hepatitis C virus. Between 5 and 10 years after HCT, serum aminotransferase elevations were seen in 57% of HCV-infected patients, without excess mortality [239, 297, 298]. In some patients, however, the duration of HCV infection before HCT can only be estimated, and the extent of fibrosis at the time of HCT unknown; this circumstance can lead to progressive liver disease that becomes apparent following HCT [288, 289, 299]. In the time period from 10 to 40 years after HCT, HCV is the leading cause of cirrhosis, and the time to cirrhosis is shorter in HCT survivors than in other patients with chronic HCV infection [299, 300]. About one-third of HCV-infected 40-year survivors of HCT develop end-stage liver disease (cirrhosis, hepatocellular carcinoma, or liver transplant). Compared with the general population, patients who survive over 10 years post-HCT have an eightfold risk of developing a new solid malignancy; the risk of HCV-related hepatocellular carcinoma is particularly elevated in this cohort. Transplant survivors with risk factors for hepatocellular carcinoma should undergo surveillance with 6 monthly liver ultrasound scans according to international guidelines [301, 302]. Partial liver transplant from the original hematopoietic cell donor has been described [303, 304]. All HCV-infected long-term survivors should be offered DAA therapy to delay the development of cirrhosis and prevent long-term consequences of chronic HCV infection (lymphoma and other lymphoproliferative disorders) [240, 305, 306].

Hepatitis B virus. The serologic pattern of HBV infection may be atypical in HCT survivors as a consequence of immunosuppression. Clearance of HBsAg may be observed, and is particularly likely if the donor was anti-HBs positive because of prior HBV infection [261]. Patients who remain HBsAg-positive after HCT are at risk of flares of hepatitis activity, particularly at times of reduction of immunosuppression; these patients should be taking oral antiviral agents such as tenofovir or entecavir. All long-term survivors with chronic hepatitis B should be regularly monitored to assess virologic and disease status, and the need for antiviral therapy [307–309]. Long-term survivors with chronic hepatitis B do not seem to have an increased rate of progression to cirrhosis compared with non-HCT patients, and effective antiviral treatment should essentially prevent any disease progression. However, HBV viral status should be reassessed prior to reintroduction of chemotherapy [240, 310, 311]. Biologic agents, such as rituximab used in the treatment of B-cell malignancy, have a particularly high risk of reactivation of occult hepatitis B (HBsAg negative and anti-HBc positive), and prophylactic antiviral therapy is recommended [240].

Hepatitis E virus. Hepatitis E virus (HEV) is one possible explanation for enigmatic elevations of serum ALT in long-term survivors, but has been little studied. HEV can cause chronic infection in HCT survivors who remain on immune suppressive drugs because of chronic GVHD [17, 269, 296]. Oral ribavirin has been shown to be effective therapy in other situations [58].

36.3.2 Bacterial Infections

Pyogenic liver abscesses and granulomatous hepatitis caused by mycobacteria are rare after HCT. In contrast, bacterial infections of the biliary tree are not rare, owing to a high prevalence of gallbladder microlith and stone formation, leading to bactibilia and the potential for cholecystitis and cholangitis [280, 312].

36.3.2.1 Bacterial Infections Before Transplant

Liver disease is a common complication of oncology practice [310]. The most common liver presentation of bacterial infection is not liver infection per se, but the effect of inflammatory mediators (interleukin-6, TNF-alpha) on hepatocyte transporters of conjugated bilirubin (cholangitis lenta, also known as hyperbilirubinemia related to infection) [221]. Liver abscesses and biliary infections are almost always overt illnesses; more subtle presentations can be seen with granulomatous liver disease caused by mycobacteria, nocardia, and a long list of rarer organisms. Cholecystitis and cholangitis, usually related to stones, sludge, and bactibilia, must be dealt with before the start of conditioning therapy.

36.3.2.2 Bacterial Infections Through the First Year

Bacterial liver abscesses are rare in HCT recipients; however, latent mycobacterial infection (including bacille Calmette-Guerin) may reactivate within the liver with prolonged immunosuppressive therapy [313, 314]. Disseminated clostridial infection and gall bladder infection with gas-producing organisms may lead to air in the liver and biliary system. Biliary sludge (calcium bilirubinate and crystals of calcineurin inhibitors) is very common in the weeks following HCT [315]. Passage of sludge down the bile duct may cause epigastric pain, nausea, abnormal serum liver enzymes, "acalculous" cholecystitis, acute pancreatitis, and bacterial cholangitis [280, 316]. Acute cholecystitis is frequently without larger gallstones [317]. Cholecystitis in this setting may also be due to leukemic relapse with gall bladder involvement or infection by CMV, fungi, rarely by other organisms such as Hemophilus influenza, and occasionally by mucosal edema caused by acute GVHD. Biliary obstruction is a rare event, caused by a variety of disorders (common bile duct calculi or inspissated biliary sludge; GVHD of the ampullary mucosa; lymphoblastic infiltration of the common bile duct, gall bladder, and ampulla of Vater in EBV lymphoproliferative disease; CMV-related biliary disease; dissecting duodenal hematoma complicating endoscopic biopsy; and leukemic relapse [chloroma] in the head of the pancreas) [280, 317]. In patients undergoing autologous HCT, biliary strictures are commonly due to recurrent malignancy [318]. Endoscopic retrograde cholangiopancreatography is indicated in patients with clinical evidence of bacterial cholangitis and radiologic evidence of biliary obstruction and allows for biliary stenting or dilatation with acceptable risk [317, 318].

36.3.2.3 Bacterial Infections in Long-Term Transplant Survivors

Long-term survivors have an increased incidence of gallstones related to the formation of calcium bilirubinate microliths (biliary sludge) following myeloablative conditioning therapy [312, 315]. Biliary sludge, gallstones, and crystals of calcineurin inhibitor medications may cause cystic duct obstruction, common bile duct obstruction, acute pancreatitis, and their associated bacterial cholangitis and biliary sepsis [280].

36.3.3 Fungal Infections

Fungal liver abscesses were formerly very common, difficult to prevent, and treatable only with an amphotericin B–bile salt formulation that made patients ill and caused renal failure [319]. Now, pretransplant screening and antifungal prophylaxis throughout the HCT process has almost eliminated fungal liver abscesses and biliary fungal infections as major problems [320]. Use of toxic amphotericin formulations have been largely abandoned in favor of far less toxic azole, echinocandin, and liposomal amphotericin drugs. Fungal liver abscesses present with fever, tender hepatomegaly, and increased serum alkaline phosphatase levels; resistant *Candida* species or molds should be suspected if abscesses occur despite prophylaxis [320]. High-resolution CT scan or MRI may demonstrate multiple fungal abscesses, and serologic tests for fungal antigens may be useful for diagnosis [321]. However, the sensitivity of imaging tests for military fungal lesions is poor [322]. Return of neutrophil function after HCT can effect resolution of previously treatment-refractory Aspergillus infection [323]. Non-sterile herbal remedies contaminated by molds and ingested may lead to liver abscesses in survivors [324].

36.3.4 Differential Diagnosis of Hepatobiliary Problems in HCT Patients

After transplant, patients with liver abnormalities have infection as the cause far less commonly than noninfectious causes. Occam's Razor is frequently disposable in the transplant setting, as there are often multiple simultaneous causes of liver abnormalities. Table 36-2 provides an overview of hepatobiliary problems after HCT.

TABLE 36-2. Hepatobiliary diseases after hematopoietic cell transplant [5, 242, 310]

Disease	Frequency	Timing	Diagnosis	Treatment	Prevention
Sinusoidal obstruction syndrome	<10% (regimen-dependent)	Onset before day +20	• Hepatomegaly, ascites, weight gain, jaundice, multiorgan failure in severe cases • Imaging • WHVPG, histology • Note atypical presentations (acute hepatitis, anasarca)	• None proven • Defibrotide (uncontrolled observations)	• Assess patient risk before conditioning starts • Choose "liver-friendly" conditioning regimens
Cholestasis of sepsis (cholangitis lenta)	Common in neutropenic patients	Following sepsis or neutropenic fever	• Exclude other causes of cholestasis • Inferential diagnosis	Treat underlying infection	• Infection prophylaxis or expectant treatment • Ursodiol
Acute GVHD	• ~20% of allograft recipients • Rare after autograft	Day +15 to 100	• Confirm GVHD in skin, gut • Exclude other causes of cholestasis • Histology	• Prednisone 2 mg/kg/day • Ursodiol	• Optimal donor selection • Complete GVHD prophylaxis • T cell depletion protocols • Ursodiol
Acute viral hepatitis	Uncommon when prophylaxis is used against herpesviruses, hepatitis B	HSV, day +20 to 50 Adenovirus, day +30 to 80 VZV, day +80 to 250 HBV and HCV, during immune reconstitution	• Pretransplant serology and PCR results • PCR of serum for specific viruses • Liver histology/PCR/immunostains now uncommonly needed	• HSV, VZV: acyclovir • Adenovirus: cidofovir • HBV: entecavir or tenofovir	• HSV and VZV infection: acyclovir prophylaxis for all patients • If patient is at risk for HBV infection: entecavir, choose HBV immune donor • Avoid MMF in patients with HCV infection • Consider treatment of HCV with DAA drugs before conditioning
Fungal abscess	Rare when prophylaxis is used	Day 10–60	• Hepatic pain, fever • Liver imaging • Serum fungal antigen	• Antifungal drugs	• Pretransplant screening • Oral antifungal prophylaxis for all patients
Drug-liver injury	Common	Any time	• Clinical evidence	• Discontinue drug	• None
Ischemic liver disease	Confined to patients with septic or hemorrhagic shock or respiratory failure	Day 0–50	• Clinical evidence	• Restore cardiac output, blood oxygenation	• Early treatment of sepsis, bleeding
Biliary obstruction	• Transient biliary sludge, common • Stones, chloromas rare	Day 15–60	• History, examination • Biliary ultrasound	• Papillotomy ± stent if obstruction persists	• None
Idiopathic hyperammonemia	• Rare	Day 10–50	• Venous blood ammonia	• None proven	• Unknown, probably genetic defect
Chronic hepatitis C	• Formerly common	After day 80	• HCV RNA in serum • Elevations of serum AST, ALT after immune reconstitution	• DAA HCV therapy after full immune reconstitution	• Screen hematopoietic cell donors • Consider treatment of HCV with DAA drugs before conditioning
Iron overload	Very common	Pretransplant • Long-term follow-up after transplant	• MRI specific for iron quantitation • Transferrin saturation • Marrow iron quantitation • Liver iron quantitation	• May not be necessary • Phlebotomy, chelation if iron burden is very high	• Avoid medicinal iron supplements
Chronic GVHD	Common after allografts	After day 80	• Prior acute GVHD history • Chronic GVHD in other organs • Consistent ALT, alkaline phosphatase • Histology	• Immunosuppressive drug therapy • Ursodiol	• Screening for chronic GVHD at day 80

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Part VI
Fungal Infections

37
Yeast Infections After Haematopoietic Stem Cell Transplantation

Jason A. Trubiano, Sharon C.-A. Chen, and Monica A. Slavin

37.1 Introduction

Invasive fungal disease (IFD) caused by yeasts and yeast-like pathogens complicates both autologous haematopoietic stem cell transplantation (auto-HSCT) and allogeneic haematopoietic stem cell transplantation (allo-HSCT). The most commonly isolated yeasts vary according to both geographic location and prophylaxis practices. Nonetheless, invasive candidiasis (IC), which includes bloodstream infection (candidemia) and deep-seated tissue infection, continues to be the most common yeast infection after HSCT [1]. The aetiology, risk factors, diagnostic approaches and treatments for IC, cryptococcosis and infectious due to other yeasts will be described.

37.2 Epidemiology, Risk Factors, and Clinical Features of Yeast Infections in HSCT

37.2.1 Candida Infections in HSCT

IC in haematology and HSCT patients is associated with significant morbidity, mortality and hospital/patient centred costs [2–6]. Historically IC has occurred in up to 17 % of allogeneic HSCT patients, with 11 % of infections being disseminated, but the latter has been reduced by widespread use of azole prophylaxis and changes in conditioning and transplant regimens [7]. A recent report of the incidence of IC in patients with a haematological malignancy was 1.4 cases/1000 admissions [8]. After HSCT, the incidence of IC was 1 % and 1.1 % at 6 and 12 months, respectively, from the Transplant Associated Infections Surveillance Network (TRANSNET) database from multiple US centres [6]. A similar incidence in HSCT recipients was described by The European Organization for Research and Treatment of Cancer (EORTC) with a fungaemia incidence of 1.55 % of which 90 % of infections were due to *Candida* spp. [9]. Nonetheless, in some stem-cell transplant centres low rates of IC have been demonstrated, even in absence of fluconazole prophylaxis [10]. The higher rates of IC seen in some HSCT antifungal prophylaxis studies may be attributable to differences in conditioning regimens and other local practices [11, 12].

The traditional candidemia risk factors are neutropenia, intra-abdominal source, older age, corticosteroid use, hypogammaglobulinemia, APACHE II score, total parenteral nutrition (TPN), intensive-care-unit (ICU) admission, recent HSCT (<6 months), recent abdominal surgery and antibacterial therapy [13–16]. Candidemia may result from gastrointestinal colonisation and translocation or in some cases central venous catheter (CVC) colonisation [17]. Colonisation with *Candida* species, primarily *Candida albicans*, *Candida krusei* and *Candida glabrata* occurs in approximately 8–44 % HSCT recipients and is higher in allo-HSCT than auto-HSCT but colonisation rates do not appear to reflect rates of IC [18–20]. A shift in the epidemiology of IC has been observed with non-*albicans Candida* species, especially *Candida parapsilosis*, *C. krusei* and *C. glabrata*, collectively responsible for 54–87.4 % of candidemia cases [8, 9, 14, 15, 21–25]. Specifically, *C. glabrata* and other non-*albicans Candida* spp. are more commonly found in HSCT compared to solid organ transplant (SOT) recipients [26]. In haematology patients, including HSCT recipients, *C. glabrata* is an increasingly important pathogen associated not only with the traditional candidemia risk factors but also with prior azole exposure [24, 27].

Breakthrough candidemia, defined as a positive blood culture for *Candida* species after ≥3 days of antifungal therapy [28], accounts for up to 50 % of IFD in HSCT patients. In HSCT patients, candidemia is more likely to be associated with fluconazole, echinocandin or multidrug resistance depending on prior exposure to antifungals [14, 15, 28–30]. Multidrug resistant *Candida* species are currently uncommon but associated with increased mortality [28, 29].

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Table 37-1. Yeasts causing invasive fungal disease in HSCT recipients

	Clinical syndromes							
Yeast	Fungaemia	Pulmonary infiltrate	Abdominal disease[a]	Skin and soft tissue infection	CNS disease[b]	Ophthalmic[c]	Cardiac[d]	Hepatosplenic
Candida spp.	+++	+	+++	+	+	++	+	+++
Cryptococcus spp.	+	+++	+	+	+++	+	+	+
Geotrichum spp.	++	+	+	+	+	+	+	+
Malassezia spp.	++	+	–	++	–	–	–	+
Rhodotorula spp.	++	+	+	+	+	+	+	–
Trichosporon spp.	+++	+	+	+	–	–	–	+
Saccharomyces spp.	+	+	+	+	–	–	–	–

References [39, 49–51, 54, 57, 58, 61, 65, 68, 70, 73, 166–174].

Legend: The proportion of reports for each pathogen.

–: Not reported.

+: Infrequently reported.

++: Commonly reported.

+++: Very commonly reported.

[a]Including abdominal collections.

[b]CNS, Central Nervous System—Meningeal, epidural or cerebral disease.

[c]Includes any ocular and retinal disease.

[d]Includes myocarditis, pericarditis and valvular disease.

However, overall despite shifts in the epidemiology and resistance patterns of *Candida* spp., morbidity and mortality outcome measures in candidemia have not dramatically changed [2, 9, 13, 14, 21, 23]. IC remains an independent predictor of mortality in HSCT recipients with recent mortality rates of IC estimated at 30–70 % [1, 15, 22, 31–33]. The clinical syndromes of IC in HSCT recipients are shown in Table 37-1.

Disseminated IC is rare, likely due to more widespread use of antifungal prophylaxis [34]. It is a distinct clinical syndrome of persistent fever after neutrophil recovery, raised ALP and target lesions, abscesses or nodules seen on imaging of liver, spleen, kidneys or lungs. Notably, biopsy culture is often negative although yeasts and granulomata may be seen on histology [35].

37.2.2 Cryptococcosis in HSCT

Yeast-like fungi of the *Cryptococcus neoformans* complex are ubiquitous encapsulated fungi that primarily enter hosts via the respiratory tract, with potential for dissemination especially in immunocompromised hosts. Cryptococcosis is primarily caused by *C. neoformans* and *Cryptococcus gattii,* both of which have been reported to cause infection in haematology patients. Invasive disease due to non-*neoformans Cryptococcus* spp. is rare [36, 37].

Cryptococcosis is less frequent in HSCT compared with SOT recipients [38]. In HSCT recipients it accounts for <1 % of all IFD, possibly due to use of antifungal prophylaxis [6, 11, 39–42]. In those few cases reported in HSCT, incidence appears higher in auto-HSCT than allo-HSCT [11, 37, 38, 41, 43]. Whilst pulmonary and central nervous system (CNS) disease remain the most common sites of infection, unusual presentations including panniculitis have been reported [42] (see Table 37-1).

37.2.3 Uncommon and Endemic Pathogens in HSCT

Yeasts that are infrequently encountered in HSCT recipients include *Malassezia* spp., *Rhodotorula* spp., *Saccharomyces* spp. and *Trichosporon* spp. [44]. However, some previously uncommon and more intrinsically antifungal resistant yeasts such as *Trichosporon* spp. are emerging related to patterns of prophylaxis use and may present as breakthrough infections [45–47]. They may be associated with central venous or arterial line use, particularly *Rhodotorula* spp, and occur more frequently in warm moist climates leading to geographic variations in incidence [46, 48]. The crude mortality of these emerging pathogens can be upward of 70 % [39]. *Malassezia* spp. classically causes tinea versicolor and skin colonisation. *Malassezia* spp. IFD typically occurs at 30–50 days post HSCT, resulting in pulmonary, catheter-related fungaemia and cutaneous disease [49–51]. *Malassezia* species require long-chain fatty acids for growth and therefore have a predilection for individuals receiving lipid containing intravenous parenteral nutrition and are usually susceptible to amphotericin B. Lipid enriched media may improve isolation from patient samples [52, 53]. The mortality associated with *Malassezia* infections in this population remains low [50]. *Geotrichum* spp. rarely causes invasive disease in HSCT, the reported clinical syndrome predominately fungaemia [54–57]. *Saccharomyces* spp. whilst a known gastrointestinal tract coloniser of HSCT recipients also remains an infre-

quent cause of IFD [18, 58–61]. When *Saccharomyces* spp. IFD occurs, it is often at 43–180 days infection post HSCT [61]. Whilst many clinical isolates remain susceptible to the azoles and the echinocandins, resistance to fluconazole has been reported [62, 63].

Rhodotorula spp. infections have been reported in HSCT with increased frequency in the Asia-Pacific region, typically associated with line-related fungaemia and low mortality (9–13 %). *Rhodotorula mucilaginosa (Rhodotorula rubra)* is the most commonly isolated species, followed by *Rhodotorula glutinis* and *Rhodotorula minuta* [64]. *Rhodotorula* spp. are intrinsically resistant to fluconazole, echinocandins and amphotericin B [65]. *Rhodotorula* spp. invasive disease is typically associated with neutropenia, CVC and auto-HSCT and treatment includes central line removal [66–68].

Trichosporon spp. colonises the skin and gut of humans, and in HSCT most often causes fungaemia. Invasive disease in HSCT was first described in 1977, and since then has been increasingly reported. The most frequently encountered species are *T. asahii* and *T. beiglii* [69]. *Trichosporon* spp. are usually resistant to echinocandins and less susceptible to amphotericin B than to azoles. It has a high mortality (42–76 %), improved however by azole therapy and neutrophil recovery [39, 70]. Whilst fungaemia is the most common presentation for invasive trichosporonosis, other clinical features can include skin or hepatosplenic lesions and pulmonary infiltrates [39].

Dimorphic fungal pathogens are also important pathogens to consider in HSCT recipients who have lived in or visited endemic areas. The TRANSNET endemic fungal pathogen surveillance study identified only two cases of coccidioidomycosis and four cases of histoplasmosis in HSCT recipients [71]. In this study only 9 % of endemic pathogens were from HSCT recipients, with the remainder occurring in SOT recipients [71]. Importantly infections can occur many years after HSCT and are also associated with the use of tumour necrosis factor alpha inhibitors [72]. For blastomycosis, coccidioidomycosis and histoplasmosis, isolated pulmonary disease is reported in 36 % of patients and disseminated disease in the remainder [71]. In allo-HSCT the incidence of *Coccidiodes,* in Arizona, an endemic area, was estimated at 2.6 %, associated with pulmonary involvement in 80 % and 45 % mortality [73]. In four other cases of Coccidioidomycosis in allo-HSCT the mortality was 75 % [74, 75]. Disseminated disease sites include bone marrow, liver, gastrointestinal tract, kidney, spleen, skin and nodal disease. The median time from transplantation to infection with an endemic pathogen in HSCT is estimated at 274 days. Frequent testing for infection in higher risk patients should be performed with appropriate serology and PCR and antifungal prophylaxis considered [71, 73].

Histoplasmosis has been reported in immunocompromised hosts including solid organ transplant recipients and HIV infected, but reported in only a small number of HSCT recipients, primarily allo-HSCT [71, 76–79]. It is even less reported than coccidioidomycosis [73, 75]. It is also important to note that cases have also been reported in patients who reside outside endemic areas [76]. Blastomycosis and paracoccidioidomycosis have been reported in haematology cohorts but not HSCT recipients [80].

37.3 Diagnostics

Routine culture-based diagnostics including cultures of blood, cerebrospinal fluid (CSF) and tissue biopsy where applicable are recommended for diagnosis of suspected IC and cryptococcosis, and all yeast infections. A fungal culture allows antifungal sensitivity testing to be performed. Blood cultures, although specific for invasive IC, lack sensitivity (50–83 %) overall and are limited by relatively delayed time to positivity (2–3 days) [1, 81, 82]. The time to positivity (TTP) can be a clue to *Candida* species, a TTP less than 56.5 h had a negative predictive value (NPV) of 92 % for *C. glabrata* [83]. In a single centre retrospective review the mean time to detection from blood cultures of *C. albicans* was 35.3 h and for *C. glabrata* 154 h [84]. It is important to note that whilst the isolation of *Candida* spp. from a sterile site is significant, isolation from non-sterile sites (including bronchoalveolar lavage) may reflect colonisation rather than invasive disease. The isolation of *Cryptococcus* however from any culture, even from a non-sterile site, should be considered reflective of invasive disease unless proven otherwise.

37.3.1 Non-culture-Based Diagnostics

37.3.1.1 1,3-β-D-Glucan (BDG)

BDG is a cell wall component of several fungal pathogens, including *Candida*. It however is not present in *Cryptococcus* species. BDG could be a useful test for diagnosis of IFD in HSCT recipients when combined with adjunctive clinical, radiological and microbiological findings. The majority of BDG studies use one of the four commercially available assays, based upon two-three times per week surveillance testing. BDG is now recommended by several guideline groups for the diagnosis of IC [85–95], however the utility of BDG has not been extensively studied in HSCT cohorts. A review of BDG by European Congress for Infections in Leukemia (ECIL) examined a heterogeneous group of 10 studies utilising BDG in haematology cohorts, demonstrating no clear difference between assays and variable sensitivity (50–100 %), specificity (45–100 %) and NPV (73–100 %) [87, 88]. Improvement in specificity (100 %) is demonstrated by obtaining two consecutive positive BDG results, at the expense of sensitivity (45–65 %) [89, 91, 95]. In a recent study of haematology patients including HSCT, BDG had insufficient sensitivity to detect breakthrough candidemia [96]. In a small number of case reports BDG has remained

positive after clinical resolution of IC [97, 98]. Moreover, in HSCT patients receiving concurrent immunoglobulin replacement, false positive BDG tests have been reported [99, 100]. In cases of rare invasive yeast infection, such as trichosporosis, BDG is often falsely negative [70]. We therefore suggest that BDG be used only in settings where serial testing is available and interpreted in conjunction with clinical, microbiological and radiological evidence of IFD.

37.3.1.2 T2 Diagnostics

T2 magnetic resonance (T2MR) technology is being increasingly used for the diagnosis of IC [101]. T2MR is a self-contained instrument that detects and amplifies Candida DNA from whole blood [102]. A recent multicentre study of over 1800 patient blood cultures, 250 of which were spiked with *Candida* spp., including HSCT recipients (43 % cohort), demonstrated a sensitivity of 91 %, specificity of over 98 % and NPV of 99 % for *Candida* spp. [102]. The median time to a negative result in this study was 4.4 h compared with 2–5 days for routine blood cultures [102]. Whilst T2MR remains promising, its role in HSCT centres is currently ill defined.

*37.3.1.3 **Candida** PCR*

Candida specific and panfungal PCR have been employed for the diagnosis of IC, however methodologies are yet to be standardised and validated. *Candida* PCR has been demonstrated to detect IC prior to standard culture methods, however the evidence for routine use of this diagnostic modality in HSCT and its performance in the presence of antifungal prophylaxis is absent [103, 104]. Using data extrapolated from mixed patient population studies Avni et al. demonstrated in a meta-analysis that the sensitivity and specificity of *Candida* PCR in cases of candidemia was 100 %, whilst in suspected IC the sensitivity was 91 % and specificity 95 % [104]. Others have shown that PCR is more sensitive than blood cultures for diagnosis of intra-abdominal infection, although HSCT recipients were not included in this study [105]. Greater validation is required in at risk groups such as HSCT recipients before widespread clinical use.

37.3.1.4 Cryptococcal Diagnostics

The presence of the polysaccharide capsule surrounding *C. neoformans* has led to the development of the cryptococcal antigen assays (CrAg) that detect the antigen in serum and CSF. CrAg detection is included in the EORTC/MSG criteria for diagnosis of IFD [87]. Whilst the test specificity when performed on serum or CSF is excellent (92–100 %) [106, 107], the sensitivity of CrAg whilst higher than blood cultures is lower (77–95 %) in the relatively small number of studies in haematology/HSCT patients [107–111]. Due to paucity of data regarding serum CrAg use in HSCT, it cannot be used as a stand-alone test to exclude cryptococcal disease or monitor response to therapy. Interestingly a false positive cryptococcal antigen has been reported in case of invasive trichosporonosis due to the cross-reactive capsular antigen [112].

The Cryptococcal lateral flow assay (LFA) has 97 % concordance with enzyme immunoassay antigen detection and an overall sensitivity and specificity for cryptococcosis of 97–100 % and 99.6 %, respectively [113–116]. Its rapid nature, low cost and "point-of-care" deliverability in conjunction with subsequent validation on sera and CSF specimens make it an attractive diagnostic for cryptococcal disease. Potential use of the assay on urine samples has been demonstrated primarily in HIV cohorts, allowing for earlier detection of invasive disease in the future [114, 117–119].

37.3.1.5 Matrix-Assisted Laser Desorption/ Ionisation-Time of Flight Mass Spectrometry (MALDI-TOF MS) for Yeast Identification

The use of MALDI-TOF MS has been increasingly reported for rapid identification of yeast species including directly from blood cultures [120, 121]. It detects mass patterns of organisms from clinical isolates, comparing against reference strains, allowing species identification within minutes [120]. In a small number of studies MALDI-TOF MS has been used to simultaneously identify *Candida* spp. and to determine susceptibility to triazole antifungals and the echinocandins [122, 123]. In candidemia MALDI-TOF has been successfully employed to detect fungaemia, more rapidly than methods based on culture and molecular diagnostic methods [124]. Correct identification of *Candida* spp. by MALDI-TOF has been referenced at 95–98 % [120, 121, 125]. The utility of MALDI-TOF MS in routine diagnostics in haematology patients has not been extensively validated, although remains promising.

37.4 Antifungal Resistance

Laboratory testing for susceptibility to antifungal drugs is a vital component of the diagnostic approach to invasive yeast infection. Patterns of resistance may vary between centres. Whilst *C. albicans* almost exclusively remains fluconazole sensitive, increasing resistance in non-*albicans Candida* spp. has been observed. Prior fluconazole therapy and echinocandin use correlated with fluconazole and caspofungin resistance, respectively [126]. The risk factors for fluconazole resistant *Candida* spp. in HSCT were identified to be prior fluconazole use, *C. glabrata* isolation, prior amphotericin B therapy, diabetes, CMV antigenemia and non-

Hodgkin's lymphoma on multivariate analysis of the TRANSNET study [26]. In cancer patients including HSCT and gastrointestinal surgery, triazole therapy and advanced age (age > 65) are associated with fluconazole resistant *Candida* spp. [7].

Fluconazole resistance has been increasingly noted in *C. glabrata* [1, 8, 14, 22, 26, 127, 128]. This is compounded by concomitant echinocandin resistance associated with fluconazole resistance phenotypes in some countries, independent of prior azole exposure [28, 129]. The emergence of multidrug resistant *C. glabrata*, involving azoles and echinocandins is cause for concern, associated with increased all-cause mortality [29, 129, 130]. Fluconazole susceptible dose-dependent (SDD) *C. glabrata* isolates have been successfully treated with fluconazole therapy in HSCT recipients [24, 131]. The resistance patterns of uncommon yeasts are outlined in Table 37-2.

There are no standardised antifungal susceptibility breakpoints for *Cryptococcus* spp. In many centres routine susceptibility testing is not performed considering resistance to first line therapies (amphotericin B and 5 flucytosine/fluconazole) is infrequently reported [1, 132].

37.5 Impact of Antifungal Prophylaxis on Invasive Yeast Infections Post HSCT

The widespread use of azole prophylaxis in HSCT patients has significantly reduced the incidence of IC and patient mortality (Figure 37-1) [7, 9, 133, 134]. Fluconazole prophylaxis to day 75 after HSCT has reduced candidemia and improved overall survival in HSCT recipients, even if colonised [7, 20]. When triazole or echinocandin antifungal prophylaxis is employed, the incidence of *Candida* spp. colonisation is decreased significantly [135]. There have been few head-to-head studies demonstrating efficacy of one agent over the other, with minor differences in occurrence of IC noted in a systematic review and meta-analysis [136]. Prophylaxis strategies are risk stratified in regard to known host factors, corresponding with either Candida directed prophylaxis (fluconazole) or mould active therapy (i.e. itraconazole, voriconazole, posaconazole).

Although 400 mg/day fluconazole was used in the randomised placebo controlled trials of prophylaxis [7], in one observational study low dose fluconazole therapy (200 mg) prevented *C. glabrata* or *C. krusei* infection in allo-HSCT but not autologous HSCT patients [25]. However, 200 mg or even 400 mg daily may not achieve adequate pharmacokinetic targets to treat invasive infection with less susceptible isolates [137]. Risk factors for acquisition of fluconazole resistant candidemia not surprisingly include recent gastrointestinal surgery and fluconazole/triazole exposure [14]. However, one study found no increase in the proportion of *C. glabrata* positive cultures in patients receiving fluconazole prophylaxis [135]. *C. krusei* is inherently resistant to fluconazole and is isolated twice as frequently in subjects receiving fluconazole compared to those receiving posaconazole or itraconazole [135]. Prolonged fluconazole prophylaxis in HSCT patients with GVHD is also associated with increased risk of *C. albicans* resistance [135].

Voriconazole prophylaxis was better tolerated but equally effective at reducing IFD, including IC, when compared with itraconazole (1.3 % vs. 2.1 %) in allo-HSCT [138]. *C. glabrata* and *C. krusei* breakthroughs have been noted in HSCT recipients receiving primary or secondary voriconazole prophylaxis [27, 139–141]. In myeloablative HSCT recipients at standard risk for early death or relapse, one randomised, double-blind study of voriconazole and fluconazole concluded that fungal-free survival rates (the primary endpoint) were similar at 180 days [142]. Notably, fluconazole and voriconazole were similarly tolerated with the same proportion of withdrawals due to adverse events (AEs) at a similar median time. *Candida* infections occurred in 1 % of patients on each arm [142].

Breakthrough IC, in particular that due to *C. glabrata* has also been noted in allo-HSCT recipients receiving posaconazole prophylaxis [135, 143, 144]. In some centres *Candida* spp. was the most common breakthrough IFD seen in HSCT recipients [135, 144].

Echinocandin prophylaxis in HSCT is not reported to be associated with high rates of breakthrough yeast infections [45]. Nonetheless breakthrough infections due to *Candida* spp., including *C. parapsilosis, C. tropicalis* and *C. glabrata* have been reported, even after short periods of echinocandin exposure [12, 19, 128, 145, 146].

37.6 Treatment

The treatment principles for IFD in HSCT are similar to those guidelines provided for general haematology and oncology populations [1]. Practical points and empirical treatment for IC in HSCT are outlined in Figure 37-2 and doses of antifungals commonly used to treat IC are shown in Table 37-3. Early initiation of empirical therapy, usually with an echinocandin followed by targeted step-down antifungal therapy is vital, as delayed therapy is associated with inferior outcomes [147–149]. In addition, delay in catheter removal in *Candida* blood stream infections is associated with increased mortality [150–152]. However, in neutropenia the gastrointestinal tract is a potential source of *Candida* infection and line removal may not always be required in such cases [17]. The incidence of ocular candidiasis in patients with candidemia (12.5–26 %) necessitates ophthalmologist review in all cases of candidemia [153–155]. The role of echocardiography to examine for *Candida* spp. endocarditis has long been debated. A recent prospective cohort study demonstrated an overall *Candida* spp. endocarditis

TABLE 37-2. Summary of suggested antifungals for invasive yeast infections in HSCT

Yeast	Primary clinical syndromes	First line	Second line	Minimum duration	Comments
Candida spp.	Candidemia (see Figure 2)	Echino[a]	Azole[a,b]	2W	1. Echinocandins not recommended for urinary or ocular candidiasis 2. If no surgical intervention for endocarditis, then lifelong suppressive fluconazole
	CNS (meningitis/abscess)	5FC + L-AMB	Azole[c]	4–6W	
	Retinitis	AMB or Azole[c]	L-AMB or Azole[d]	4–6W	
	Endocarditis	5FC + L-AMB	Echino + L-AMB	6W	
	Intra-abdo collection	Azole[b]	Echino[a]	2–4W	
	Hepatosplenic[e]	Azole[b]	L-AMB/Echino	16–26W	
	Cystitis/pyelonephritis	Azole[b]	L-AMB +/− 5FC	2W	
	Osteomyelitis	Azole[b] or L-AMB[a]	Echino[a]	26–52W	
	Septic Arthritis	Azole[b] or L-AMB[a]		6W	
C. neoformans	CNS	I: L-AMB +5FC	High dose L-AMB[f]	2W	1. L-AMB preferred over AMB 2. Voriconazole can be considered for salvage therapy
	Pulmonary (mild)	C: Azole[c]	–	8W	
	Pulmonary (severe)	M: Azole	–	26–52W	
		Azole[b]	–	26–52W	
		Same as "CNS"		26–52W	
C. gattii	CNS	I: L-AMB +5FC	–	6 W	1. Azole induction not recommended 2. For mild lung disease may consider azole for 6–12 months
	Pulmonary	C: Azole[b]		52–78W	
		I: L-AMB +5FC		2W	
		C: Azole[b]		26–52W	
Histoplasma spp.	Pulmonary or disseminated	L-AMB	AMB OR Azole[b,d]	4–6W (induction) 52W (consolidation)	1. Step down from L-AMB to itraconazole 200 mg bd-tds. 2. For non-CNS/non-disseminated disease can consider itraconazole first line
Coccidioidomycosis	Pulmonary or disseminated	L-AMB	AMB Azole[g]		1. For mild disease can consider fluconazole or itraconazole
Geotrichum spp.	Fugaemia and disseminated	Azole[d]	L-AMB	2W	1. Variable susceptibility to fluconazole
Malassezia spp.	Cutaneous disease	Azole[d]	Azole[b]	1–2W	1. Variable AMB susceptibility 2. No echinocandin susceptibility data 3. Intraconazole susceptible
Rhodotorula spp.	Fungaemia, pneumonia or invasive disease	L-AMB	Azole[d]	2W	1. Intrinsic resistance to fluconazole and echinocandins
Saccharomyces spp.	Fungaemia	Azole[d]	L-AMB/Azole[b]		1. Variable fluconazole susceptibility 2. No echinocandins data
Trichosporon spp.	Fungaemia, pneumonia or invasive disease	Azole[d]	Azole[b]	2W	1. Increasing rates of fluconazole and AMB resistance. 2. Limited evidence for combination therapy 3. Echinocandins have poor activity.

Adapted from references [1, 39, 51, 54, 56, 65, 68, 70, 71, 73, 161, 164, 166, 167, 176–180].

Abbreviations: *L-AMB* liposomal amphotericin B, *AMB* Amphotericin B, *Min duration* minimum treatment duration, *W* weeks, *Echino* echinocandin, *5FC* 5-Flucytosine, *CNS* central nervous system, *I* induction, *C* consolidation, *M* maintenance.

[a]If echinocandin started, follow with azole if susceptible (fluconazole > voriconazole).

[b]Fluconazole preferred if susceptible.

[c]Fluconazole 800 mg OR 12 mg/kg daily.

[d]Voriconazole preferred if susceptible.

[e]Disseminated disease.

[f]Liposomal Amphotericin 6 mg/kg daily.

[g]Fluconazole or voriconazole preferred.

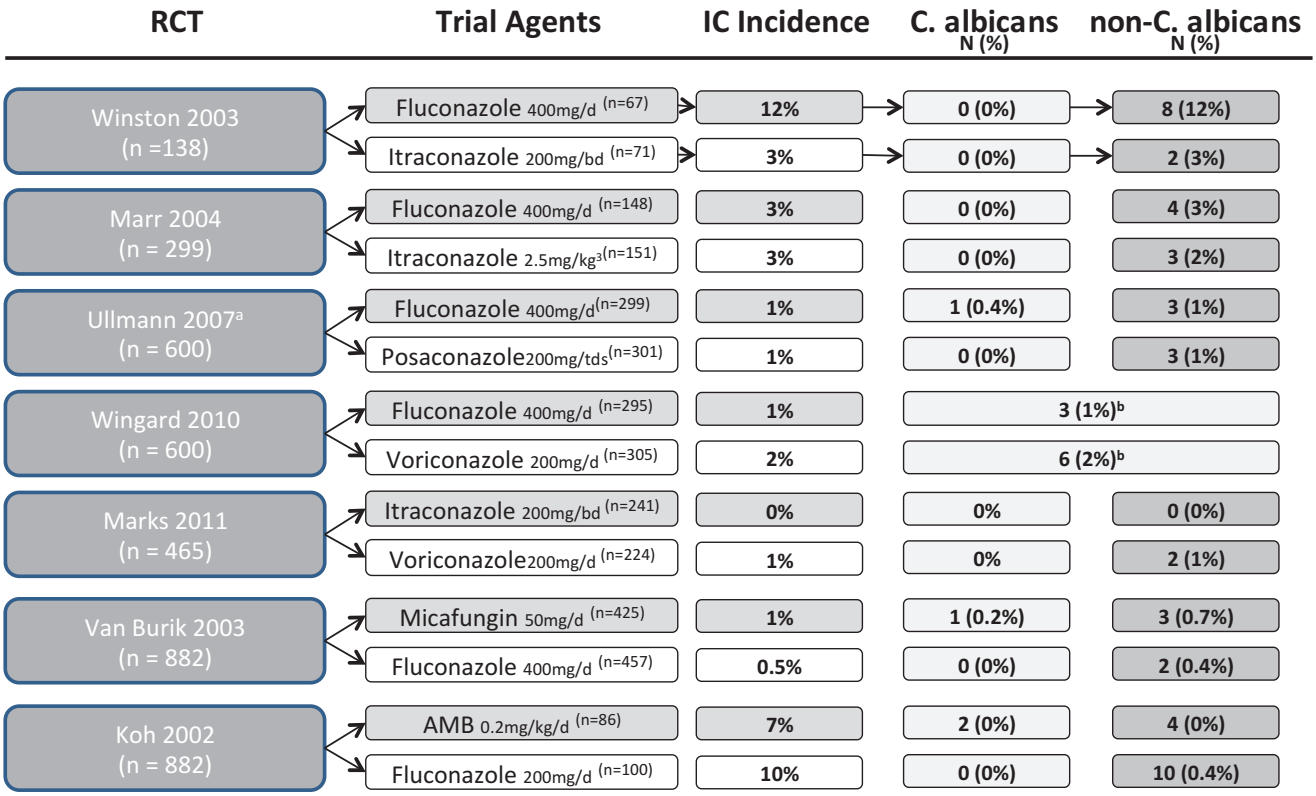

FIGURE 37-1. The incidence of IC in seven contemporary randomised control trials of antifungal prophylaxis in allo-HSCT recipients. *Abbreviations*: *RCT* randomised control trial, *N* study number, *IC* invasive candidiasis, *AMB* amphotericin B, *d* daily, *bd* twice-daily, *tds* three-times-daily. Maintenance dosing for agents listed. [a]Ullman et al. included only allo-HSCT with severe GVHD. [b]Missing information regarding *Candida* spp. References [133, 134, 138, 142, 175].

incidence of 4.2% [156]. When transoesophageal echocardiogram (TOE) is performed routinely in most centres, the incidence has been reported at upwards of 17% [157]. Echocardiography should be strongly considered, especially in the setting of prosthetic valves.

Hepatosplenic or disseminated candidiasis may require prolonged treatment and be complicated by prolonged fever. It has been suggested that this entity may represent an immune reconstitution inflammatory syndrome (IRIS) and some authors have suggested use of corticosteroid treatment [34]. IC is not a contraindication to HSCT [158]. Where neutropenia is expected to resolve granulocyte infusions have been used in conjunction with antifungal therapy to bridge a period of profound neutropenia but in the absence of a randomised trial efficacy is uncertain [159, 160].

The initial antifungal treatment choice is dependent on the causative species, site of infection, presence/absence of candidemia, host factors, prior azole exposure, and local hospital epidemiology. It is imperative to differentiate *Candida* spp. colonisation from IC, in particular isolation of *Candida* spp. from urinary and upper respiratory tract sources in the absence of clinical or radiological evidence of disease. Although *Candida* spp. colonisation, especially from multiple sites, may suggest candidemia, alone it does not warrant therapy.

The treatment of cryptococcosis in HSCT is outlined in the 2010 IDSA guidelines and is summarised in Table 37-2 [161]. The treatment in HSCT should not differ from recommended guidelines, although the data primarily stem from HIV/AIDS patients [1, 161, 162]. In all patients with cryptococcosis, a CSF opening pressure should be measured and a CSF specimen obtained for CrAg testing and culture. There is however limited evidence regarding the approach to patients who have recently completed treatment for cryptococcosis and about to undergo HSCT. From a small retrospective study, Zuniga et al. demonstrated that adequately treated cryptococcal disease was not a contraindication to transplantation [163]. For *C. gattii* infections a longer induction therapy is recommended due to risk of treatment failure [164]. In treatment of cryptococcal infections after HSCT, the possibility of IRIS with rapid reduction of immune suppression should be considered [165]. The treatment of other rare and emerging yeast infections is summarised in Table 37-2. The recommended antifungal dosing schedules are summarised in Table 37-3.

Invasive candidiasis general principles

1. Remove implicated central venous catheter in cases of candidemia
    a. If catheter or device CANNOT been removed consider echinocandin (biofilm activity)
2. Avoid delays in empirical antifungal therapy - Delays associated with increased mortality
3. Avoid using an echinocandin alone in patients with evidence of retinal candidiasis[a]
4. Renally excreted agents more effective in patients with Candida pyelonephritis, cystitis or fungal ball[b]
5. Strongly consider ophthalmic examination and echocardiography in all candidemia patients
6. Repeat blood cultures to ensure fungaemia clearance on therapy and determine duration of therapy

Considerations when choosing Empirical treatment for candidemia

1. Tailor empirical therapy to local epidemiology
2. Consider patient clinical status and prior antifungal exposure and colonisation:
    a. If patient haemodynamically stable & non-neutropenic, treatment choices include:
        i. Echinocandin (micafungin, caspofungin or anidulafungin)
        ii. Voriconazole
        iii. L-AMB
        iv. Fluconazole[c]
    b. If haemodynamically unstable OR neutropenic, or recent azole exposure or prophylaxis treatment choices include:
        i. Echinocandin (micafungin, caspofungin or anidulafungin) -
        ii. L-AMB
        iii. Voriconazole
    c. If recently colonised with Candida spp. consider its known susceptibility when employing empirical therapy for presumed invasive candidiasis.
    d. If recent echinocandin exposure or concerns with echinocandin resistance
        i. L-AMB
        ii. Voriconazole

Abbreviations: L-AMB, liposomal amphotericin B
[a]An azole such as fluconazole or voriconazole achieves levels in vitreous fluid but echinocandins do not
[b]Fluconazole & 5-flucyotsine are renally excreted whilst echinocandins are not
c 12mg/kg loading then 6mg/kg ongoing

FIGURE 37-2. Practice points and empirical therapy for invasive candidiasis and candidemia in HSCT. *Abbreviations*: *L-AMB* liposomal amphotericin B. [a]An azole such as fluconazole or voriconazole achieves levels in vitreous fluid but echinocandins do not. [b]Fluconazole & 5-flucyotsine are renally excreted whilst echinocandins are not. [c]12 mg/kg loading then 6 mg/kg ongoing.

TABLE 37-3. Treatment doses for commonly used antifungals in invasive candidiasis

Agent	Dose & frequency	Route
Fluconazole[a]	Loading—12 mg/kg OR 800 mg daily (single dose) Maintenance—400 mg OR 6 mg/kg daily	IV or oral
5-Flucytosine[a,b]	Loading—Nil Maintenance—25 mg/kg four-times-daily	IV or oral
Liposomal amphotericin	Loading—Nil Maintenance—3–5 mg/kg daily	IV
Voriconazole	Loading—6 mg/kg twice-daily for 2 doses Maintenance—4 mg/kg twice daily	IV or oral
Caspofungin	Loading—70 mg daily (single dose) Maintenance—50 mg daily	IV
Micafungin	Loading—Nil Maintenance—100 mg daily	IV
Anidulafungin	Loading—200 mg daily (single dose) Maintenance—100 mg daily	IV

[a]Require dose adjustment in renal impairment.
[b]Suggest performing trough levels to achieve clinical efficacy (>25 mg/L) and peak levels to avoid toxicity (<100 mg/L).

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38
Yeast Infections After Solid Organ Transplantation

Todd P. McCarty and Peter G. Pappas

38.1 Candida

38.1.1 Epidemiology

Candida is a genus of yeast found in abundance worldwide. There is a wide variety of species found throughout the environment, however only about 15 of these are commonly pathogenic in humans. In addition to causing disease, the yeast can be found throughout the body as a part of the normal human microbial environment.

The Transplant-Associated Infection Surveillance Network (TRANSNET) study reported in 2010 that *Candida* comprised more than half of all documented invasive fungal infections in SOT recipients [1]. *Candida albicans* was the predominant species, however was the etiologic pathogen in approximately 50% of *Candida* cases. *C. glabrata* comprised a quarter while the remaining cases were primarily caused by *C. parapsilosis*, *C. tropicalis,* and *C. krusei*. Polymicrobial infection occurred in almost 10%. The Prospective Antifungal Therapy (PATH) registry followed a broader group of patients and saw a similar distribution of species overall, however when looking at solely solid organ transplant recipients, observed *C. glabrata* to be the most common species at nearly 40% [2]. They also demonstrated similar findings of *Candida* being the most common cause of fungal infection in solid organ transplant (SOT) recipients [3]. A follow-up study from the PATH registry looking solely at non-albicans species observed *C. glabrata* to cause over 60% of non-albicans candidiaisis (Table 38-1) [4]. A similar population-based monitoring program, the SENTRY Antimicrobial Surveillance Program, demonstrated *C. albicans* as the most common with *C. glabrata* second, however at a lower frequency (less than 20%) compared to TRANSNET and PATH Alliance [5]. One key difference between the studies, however, is that while TRANSNET and PATH were confined to North America, SENTRY was a worldwide study. The SENTRY breakdown by region shows their North America rates by species to be similar to the other studies, with much lower rates of *C. glabrata* in the other parts of the world [6]. *C. parapsilosis* supplants *C. glabrata* as the second most common species in their Latin America isolates.

38.1.2 Pathogenesis

As the most common species, the majority of work looking at pathogenesis and virulence has focused on *C. albicans. C. albicans* has an ability to exist along a spectrum from budding yeast to a walled hyphal structure [7]. One primary mode of virulence is the ability of the yeast to adhere to surfaces, including human cells, and convert to the hyphal form for the purposes of invading tissue [7]. Indeed, altering genetics to prevent the transition from yeast to hyphal phase has been shown to decrease pathogenicity [8]. The ability to adhere to surfaces is also an important contributor to human disease with the ability to form biofilms on prosthetic surfaces, including a concurrent upregulation of resistance mechanisms [9]. The adherence of fungal cells to a surface and formation of a biofilm prompt the development of "persister cells" that are highly resistant to antifungals [10].

38.1.3 Clinical Manifestations

38.1.3.1 Superficial Infections

As SOT recipients have their immune system influenced by pharmacologic immunosuppression, in particular glucocorticosteroids, *Candida* is presented with the opportunity to transform from commensal to pathogen. The spectrum of superficial disease ranges from cutaneous to mucous membrane and can occur in a variety of sites. Additional co-morbid conditions can contribute to the development of oropharyngeal thrush or vaginitis, such as concurrent antibiotic use (e.g., prophylaxis) and diabetes. Oropharyngeal thrush can progress to a more invasive form of mucosal disease, specifically,

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Table 38-1. Species distribution in candidemia among patients with solid organ transplants

	TRANSNET	PATH alliance
Species	*N*=264	*N*=292
C. albicans	131 (50%)	97 (33%)
C. glabrata	78 (30%)	112 (38%)
C. parapsilosis	23 (9%)	33 (11%)
C. tropicalis	12 (5%)	16 (5%)
C. krusei	14 (5%)	8 (3%)

Candida esophagitis. A more severe infection such as this must be dealt with promptly as potential complications ranging from stricture to perforation and death have been reported [11, 12].

38.1.3.2 Candidemia

Candidemia is the most common manifestation of invasive candidiasis amongst transplant recipients based on data from the TRANSNET cohort [1, 13]. The following risk factors are well understood to place patients at risk for invasive candidiasis: neutropenia, chemotherapy, colonization with *Candida*, broad-spectrum antibiotics, central venous catheter, hemodialysis or renal failure, critical illness, parenteral nutrition, mechanical ventilation, surgery, and advanced age [14]. It is not uncommon for the SOT recipient to meet one or more of these factors.

Liver transplant recipients are at particularly increased risk of candidemia with a variety of potential factors taking into account pre- and post-transplant variables. Using a focused algorithm of creatinine greater than 3 mg/dL, transplant operative time greater than or equal to 11 h, retransplantation, receipt of more than 40 units of blood products, or early fungal colonization, the presence of two or more factors identified a group of patients in whom 67% developed an invasive fungal infection with *Candida* being the most common genus [15]. Since the establishment of the Model for End-stage Liver Disease (MELD), this has now been evaluated for its contribution to predicting infectious complications [16, 17]. In multivariate analysis including other known risk factors for invasive fungal infections (IFIs), an elevated MELD score has been shown to have increased odds for developing all types of IFIs including invasive candidiasis and candidemia [17].

38.1.3.3 Urinary Tract Infection

Candida is an uncommon pathogen in the urinary tract; however, it is a frequent colonizer in certain patients. Patients with urinary catheters, diabetes, on broad-spectrum antibiotics, or prolonged hospitalization are all prone to *Candida* isolation from urine culture specimens. Renal transplant recipients, in particular, pose a dilemma over what to do with positive culture results in the setting of manipulation of the urinary tract and possibly the placement of a ureteral stent. Prosthetic materials are one of the situations where *Candida* can evolve from colonizer to pathogen. Studies to determine the true incidence of candiduria in renal transplant recipients are inconclusive, however it probably approximates 10%, not dissimilar to the hospitalized population as a whole [18, 19]. These studies have failed to show a substantial benefit to treating a positive *Candida* urine culture in the absence of symptomatic probable or proven disease. Infection can be severe with pyelonephritis having been reported [20, 21].

38.1.3.4 Intra-Abdominal

Candida has long been known to be a colonizer of the gastrointestinal tract, and therefore controversy has persisted over whether its presence in peritoneal culture represents colonization versus invasive infection [22]. In particular, complicated nosocomial peritonitis appears to be an instance of true infection [23]. Liver, small bowel, and pancreas transplant recipients can be of increased risk and, if certain criteria are met, may warrant fluconazole prophylaxis to prevent invasive candidiasis. This will be discussed in more detail below.

38.1.3.5 Pulmonary

Candida as a cause of primary pneumonia is exceptionally rare. While pulmonary disease does occur, it is generally in the form of hematogenous spread from other sources. This generally appears radiographically as septic emboli. *Candida* frequently occurs as a colonizer either of the respiratory tract or, in the mechanically ventilated, the endotracheal tube given the organism's propensity to adhere to surfaces. Studies have failed to find an association between microbiologic growth of *Candida* from bronchoscopic specimens and an impact mortality or other outcomes [24, 25]. An exception to this statement is limited to anastomotic tracheobronchitis in lung transplant recipients. This is a well-described entity and can be caused by a wide variety of organisms, including *Candida* [26]. A single center study looking at the causes of tracheobronchitis in 272 heart–lung or lung recipients found 15 anastomotic infections, of which *Candida* was the most common pathogen, having been diagnosed in eight of the patients [27].

38.1.3.6 Ocular

Involvement of the eye is an uncommon but well-recognized complication of disseminated invasive candidiasis. Clinical manifestations range from chorioretinitis to full-blown endophthalmitis. Rates as high as 26% for ocular spread associated with candidemia have been reported. Historically *C. albicans* is more likely than other species to cause ocular involvement based on its innate invasive potential, while *C. parapsilosis* is the least likely compared to other species [28, 29]. There are no prospective studies looking at rates of ocu-

lar candidiasis in solid organ transplant recipients; however, it has been reported in the SOT population [26, 30, 31]. It does appear to be an uncommon complication, as one series reporting all ocular infections from a cohort of heart transplant recipients had no cases caused by *Candida* [32].

38.1.3.7 Donor-Derived

Presence of *Candida* in the gastrointestinal tract raises the potential for transmission in the process of the intra-peritoneal organs [33]. Additionally, the presence of candidemia prior to or at the time of death of the donor would raise the potential for transmission. While not strictly a donor-derived complication, there are numerous case reports of *Candida* contaminating the preservation fluid in transport from donor to recipient [34–39]. A study of graft-site candidiasis deriving from organ recovery in renal transplant recipients found renal arteritis to be the most common complication [40].

38.1.4 Diagnosis

Culture is currently the gold standard of diagnosis. While it is difficult to interpret a culture positive for *Candida* species from a non-sterile site, sterile cultures are indicative of an invasive process and should be treated with the utmost urgency. Given the benefits of early and effective treatment and the variation of anti-fungal sensitivities based on species, efforts are underway to develop more reliable means of rapid diagnosis and species identification.

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) is one means of rapid identification that can lead to faster adjustment of antifungal treatment. MALDI-TOF has been shown to identify *Candida* species with accuracy equal to or greater than conventional methods [41]. Additionally, the technology has been shown to drastically decrease time to identification, in particular for the non-albicans *Candida* species [42]. To run the assay, a pure culture specimen must undergo preparation specific to the brand of MALDI-TOF in use. While this earlier identification can be beneficial in making empiric adjustments in treatment, it does not impact the time to diagnosis of invasive candidiasis since it still requires a positive culture.

Peptide nucleic acid fluorescent in situ hybridization (PNA-FISH) can be performed directly from a positive blood culture bottle prior to being plated for isolation of pure colonies with a very high level of sensitivity and specificity for *C. albicans* [43]. Commercially available multi-species kits now exist but have some limitations in their ability to distinguish completely to the species level with pairing of species to a single color fluorescence [44]. The test can be run directly from positive blood culture bottles or from subcultured colonies. While this has the potential to provide some identification data even faster than MALDI-TOF, it is still reliant on *Candida* growing in culture.

Table 38-2. Performance of non-culture based diagnostic assays for candidemia and/or invasive candidiaisis

Assay	Sensitivity	Specificity
(1 → 3)-β-D-glucan	57–97%	44–93%
PCR	73–93%	90–96%
T2Candida®	88–94%	98.9–99.9%

The most recent technology to become available is the T2Candida® assay. Based on magnetic resonance technology, the assay is able to identify to a paired species level (*C. albicans/C. tropicalis*, *C. krusei/C. glabrata*, and *C. parapsilosis*) from a whole blood specimen without waiting for a positive culture [45]. The technology can identify a positive signal within hours of the obtaining the whole blood specimen, thus having the potential to identify candidemia much sooner and lead to earlier initiation of appropriate therapy. Additionally it has an excellent negative predictive value for candidemia which could be used to de-escalate or stop unnecessary anti-fungal therapy very quickly (Table 38-2).

Non-fungemic invasive candidiasis remains a challenge to diagnose [46]. An assay to detect (1 → 3)-β-D-glucan (BDG), a component of the *Candida* cell wall, in serum or plasma has been shown in a meta-analysis to have a sensitivity of 76.8% and specificity of 85.3% for proven or probable invasive fungal infections from any organism [47]. Studies restricted to candidiasis have sensitivities ranging from 57% to 97% while the specificity was 44% to 93% [48]. Part of the variation in specificity is due to the presence of the protein in the cell wall of most fungi, not solely *Candida*. Thus, the assay is less specific than some other assays and is considered to be "pan-fungal" by many experts. One study restricted to liver transplant recipients showed improved performance with a sensitivity of 83% and specificity of 89% [49]. Conversely, the test performs poorly in lung transplant recipients with one study having a sensitivity of 71 and 59%, noting mold colonization of the lungs and hemodialysis raised levels of BDG [50]. A meta-analysis of polymerase chain reaction (PCR) for the diagnosis of candidiasis has shown good performance characteristics in the blood culture negative population with sensitivity ranging from 73% for culture negative candidiasis to 93% for proven/probable candidiasis [51]. Specificity for both groups was over 90%.

38.1.5 Treatment

Multiple guidelines exist to assist the clinician with ensuring appropriate treatment across the spectrum of invasive candidiasis [52–55]. Overall, treatment of the solid organ transplant is the same as treatment for the non-transplant patient. As such, the focus here will be on selected types of infection.

Candidemia initial regimen should be based on both severity of illness and the potential for a resistant isolate, in particular *C. glabrata* and *C. krusei*, but most experts agree that

echinocandins are preferred as initial therapy for most patients with candidemia [55]. For empiric therapy, echinocandins are generally preferred in the critically ill, those with a recent history of or present fluconazole use, or colonization with a resistant isolate. The TRANSNET study, performed between 2000 and 2006, documented an overall fluconazole resistance rate at that time of 16%, with *C. glabrata* and *C. krusei* comprising 30% of all isolates [13]. Lacking those risk factors, fluconazole is a reasonable option for empiric therapy in the non-acutely ill person. Once the species and susceptibilities are determined, targeted therapy can be chosen. Treatment should continue for at least 14 days from the first negative culture with an attempt to influence source control, in particular the removal of intravascular catheters [55].

For candiduria, the decision to treat should be on the basis of symptoms and/or findings consistent with a urinary tract infection. If the patient is asymptomatic but neutropenic or undergoing a urologic procedure, then treatment is warranted. The kidney(s) should be assessed for the presence of a fungal ball and surgical removal of the obstruction pursued if found. Echinocandins are poorly excreted into the urine, therefore fluconazole is the treatment of choice for most *Candida* urinary tract infections. If a fluconazole-resistant isolate is isolated, then a lipid amphotericin-B preparation can be used, with or without flucytosine [55].

Ocular candidiasis is another circumstance where echinocandins fall short in their ability to penetrate a particular tissue. Again, lipid amphotericin-B products and fluconazole are the agents of choice. Consultation with ophthalmology should be obtained early to assess the need for aggressive surgical intervention with vitrectomy [55].

Treatment of end-organ infection, such as pulmonary, intra-peritoneal, or cardiovascular candidiasis should be driven by species identification and susceptibilities. Duration of therapy will need to be tailored to the individual patient on the basis of ability to drain the infected material and reverse the source of contamination.

There is emerging evidence of the development of resistance to echinocandins, in particular in *C. glabrata. C. parapsilosis* has been noted to have, on average, higher minimum inhibitory concentrations against the echinocandins, but there is little correlation to these values and clinical response to therapy with echinocandins [56–60]. Rates of resistance appear to vary significantly across centers. The presence of azole and/or echinocandin resistance should be explored among patients failing to respond as expected to either of these therapies.

The other aspect of treatment is monitoring for drug–drug interactions (DDIs). While the echinocandins have minimal DDIs, fluconazole and other azole agents are well documented to have many potential DDIs, and care should be taken to adjust immunosuppressant dosing, especially with use of the calcineurin inhibitors, cyclosporine and tacrolimus, and the mTOR inhibitors, everlimus and sirolimus [61, 62].

38.1.6 Prophylaxis

There is a subset of intra-abdominal transplant patients who are high risk for invasive candidiasis. Criteria to determine high-risk is best established in liver transplant recipients; defined as *Candida* colonization, 40 or more units of cellular blood products transfused, retransplantation, choledochojejunostomy, and prolonged operation, having two or more of these factors warrants prophylaxis [54]. Additionally, a MELD score >30 has been shown to increase the odds of a post-transplant infection of any type [63]. There were few documented fungal infections in this study; however, the broader use of MELD has overlapped with the growing use of anti-fungal prophylaxis. This confounds the assessment of the value of MELD as a predictor of invasive fungal infection (IFI), but nevertheless, a high MELD score should be considered a risk factor for IFI and taken into consideration when deciding to give anti-fungal prophylaxis in the early post-transplant period.

A meta-analysis of antifungal prophylaxis of any sort demonstrated a decrease in all types of candidiasis and improvement of mortality attributable to fungal infections, but without an impact on overall mortality [64]. Fluconazole has been shown to be superior to both nystatin and placebo in preventing infections caused by *Candida* and is well tolerated [65, 66]. Caspofungin has demonstrated efficacy but has not been studied in a randomized, comparative trial [67]. Anidulafungin has been shown to be equally effective for antifungal prophylaxis when compared to fluconazole in a randomized, controlled trial of 200 liver transplant recipients that met the criteria for needing prophylaxis [68]. A 2008 survey of liver transplant centers in North America showed three quarters of programs used targeted prophylaxis among high-risk recipients, and fluconazole was the most commonly used agent [69]. Prophylaxis should be discontinued no more than 4 weeks after transplant unless there are ongoing concerns for invasive candidiasis.

There are no clinical trials to assess the role of antifungal prophylaxis in small bowel transplants; however given its presence as a colonizer in the gastrointestinal tract and high rate of infection, fluconazole is commonly used for this purpose [70]. Pancreatic transplantation also carries a high rate of fungal infection with one study showing the benefit of fluconazole prophylaxis on decreased candidiasis and infection free survival [71].

38.2 Cryptococcus

38.2.1 Epidemiology

Cryptococcus is an encapsulated budding yeast capable of causing a disease with a variety of manifestations. *Cryptococcus neoformans* has long been the predominant disease causing species, but the emergence of *Cryptococcus gattii* throughout the world is becoming a formidable challenge.

Originally recognized in Australia and Papua New Guinea, *C. gattii* has now been reported across the globe [72].

Comprising 8% of all IFIs in the TRANSNET dataset, the 146 cases of cryptococcosis were the third most common fungal pathogen in solid organ transplant (SOT) recipients [1]. The incidence of cryptococcosis in that study was approximately 0.2% of all solid organ transplant patients. Literature reviews of reported cases have shown a much higher incidence in SOT recipients, ranging from 1.56 to 2.8, but these represent cumulative estimates, whereas the TRANSNET data are based on calculated annual incidence [73, 74]. Cryptococcosis is rare in stem cell transplant recipients. The TRANSNET study identified only 6 cases among 16,200 enrolled stem cell transplant recipients [75]. While infection can occur any time after transplant, multiple studies show a median time to infection of 19–21 months post-transplantation [1, 74, 76]. Infection in the first month raises the possibility of pre-existing infection in the recipient or donor-derived infection [77, 78].

38.2.2 Pathogenesis

Primary infection in humans occurs through inhalation of infectious particles, though uncertainty remains over just what type of particle begins the cascade that ultimately leads to active disease. Current data suggests humans are exposed at a high rate at a young age with the organism remaining dormant for a prolonged period of time before later causing disease [79–81]. This is not uniform worldwide, however, as a study of exposure rates in children from the Philippines and two regions of New York demonstrated high variability in serologic positivity among children from the Bronx, NY, Dutchess County, NY and the Philippines [82]. A study to determine pre-transplant exposure to *Cryptococcus* in SOT recipients who were diagnosed with disease exhibited evidence of antibody responses in 52% [83]. That group also developed cryptococcosis much earlier in the post-transplant period, 5.6 months from the time of transplant rather than 40.6 months in the group that did not have evidence of pre-transplant antibodies against *Cryptococcus*. These data suggest that most cases of post-transplantation cryptococcosis are due to a reactivation event.

The polysaccharide capsule plays a key role in its ability to cause disease and evade the host immune system. It has been shown to inhibit phagocytosis and reduce the production and effectiveness of the innate immune response, including cytokines and the complement pathway [84]. Once phagocytosed and intracellular, the capsule enhances the ability of the yeast to survive oxidative stress [85]. Inoculation of a mouse with a capsule deficient strain of *Cryptococcus* leads to an increased inflammatory response and minimal production of invasive disease compared to a capsular *Cryptococcus* strain [86]. Further evidence of the importance of the capsule in the virulence of *C. neoformans* is found in the animal model. When one deletes the *CAP59* gene responsible for capsule formation, this causes a loss of virulence in a mouse model of cryptococcal meningitis; the transformation of the avirulent strain with a plasmid containing the *CAP59* gene restores its virulence in this model [87].

38.2.3 Clinical Manifestations

Cryptococcus causes a variety of clinical syndromes. In the solid organ transplant population, there is a higher frequency of isolated pulmonary disease when compared to the human immunodeficiency virus (HIV) population [88]. Disseminated disease remains a frequent occurrence with the bloodstream, central nervous system (CNS), skin and soft tissue, and urinary tract as potential sites of disease.

38.2.3.1 Pulmonary

As the primary site of most infections, the lungs are the most common site of disease, and patients can present either with primary or reactivation disease. Pulmonary disease in the SOT population can range from an asymptomatic nodular infiltrate to lobar consolidation, a mass-like infiltrate, cavitary disease, or diffuse nodular infiltrates [89, 90]. Studies of cryptococcosis in SOT recipients have found that 25–39% of cases will have disease limited to the lungs [1, 88, 89]. Higher doses of steroids have been associated with a higher rate of symptomatic and disseminated diseases [89, 91]. Duration of symptoms prior to diagnosis spans a wide range, with one study of solid organ transplant recipients reporting the presence of symptoms from 1 to 97 days [92]. Severity of illness can range from asymptomatic disease to fulminant respiratory failure [93]. Compared to HIV patients, SOT recipients have a higher rate of pulmonary disease with less CNS involvement [88]. However, the diagnosis of pulmonary disease should prompt a search for infection elsewhere, in particular a lumbar puncture to assess for involvement of the CNS [94].

38.2.3.2 Central Nervous System

As noted above, central nervous system involvement occurs at a lower frequency in SOT patients compared to HIV patients, but it remains the most common site of extrapulmonary involvement among SOT patients with cryptococcosis, occurring in 44–62% of these patients [1, 88, 95, 96]. While presenting symptoms vary, asymptomatic meningeal disease likely occurs rarely, patients can present acutely with symptoms occurring for only 2 days to several weeks [97]. Abnormal mental status, fungemia, late-onset disease (defined as more than 24 months post-transplant), and a serum cryptococcal antigen titer >1:64 have been found to be associated with an increased risk of CNS involvement [95].

Focal parenchymal lesions with or without evidence of meningitis may occur in SOT recipients [98]. Focal parenchymal lesions without meningitis are an uncommon manifestation of disease in this population. In one study, 10% of patients had focal parenchymal lesions while 13% had meningeal enhancement. Additionally, the presence of a focal meningeal lesion was associated with higher CSF cryptococcal antigen titers compared to parenchymal lesions [98]. Finally, among those with CNS infections, the presence of abnormal neuroimaging findings at diagnosis was more likely to meet the diagnostic criteria for immune reconstitution inflammatory syndrome later in their treatment course [99].

38.2.3.3 Bloodstream Infection

Rates of blood culture positivity indicating disseminated disease vary, in part due to a lack of consistent collection of the blood cultures. One study of 178 cases had 38% of blood cultures grow *Cryptococcus*, however cultures were only collected on 39 patients [74]. Other studies have shown lower rates of bloodstream infection, with the Cryptococcal Collaborative Transplant Study Group showing 21% overall but significantly more common in CNS disease with 36% of patients who were fungemic [76, 98]. Positive blood cultures were also associated with increased 90-day mortality [76]. A separate single-center study of cryptococcal meningitis in SOT recipients demonstrated similar observations with 39% of patients with CNS disease having positive blood cultures [97].

38.2.3.4 Skin and Soft Tissue

Cutaneous and subcutaneous infection is the third most common form of cryptococcosis in the SOT population, comprising 10–18% of cases [88, 100]. Appearance varies greatly, from cellulitis to abscess and ulcer formation to deep nodular and panniculitis lesions having been reported in a variety of anatomic locations [100]. Clues that should make the clinician suspect cryptococcosis rather than a bacterial etiology should include the following: bilateral or disseminated lesions, a nodular component to palpation or appearance, atypical anatomic location, tissue necrosis, and failure to respond to conventional anti-bacterial agents.

38.2.3.5 Urinary Tract

The prostate and urinary tract are known reservoirs for fungal infections, however reports of cryptococcal infection there in SOT patients are rare. Involvement of the prostate and the kidney have both been reported [101, 102].

38.2.4 Diagnosis

Multiple reliable means of establishing a diagnosis of cryptococcosis exist: cryptococcal antigen assay of bodily fluid (primarily serum and cerebral spinal fluid), routine and fungal culture, and characteristic findings on histopathology and/or cytology of pathologic samples.

38.2.4.1 Cryptococcal Antigen

A variety of types of antigen assays to detect the capsular polysaccharide antigen exist. The most experience has been developed with latex agglutination and enzyme immunoassays, although more recently a lateral flow assay with potential utility as a point-of-care test has been developed [103–105]. All methods display a high degree of sensitivity and specificity, approaching 100% depending on the sample type and clinical syndrome. One group in whom the assay performs less well is the lung transplant population, where the serum assay may have decreased sensitivity [89, 106].

38.2.4.2 Culture

Cryptococcus species do not require specialized media for reliable culture, growing readily on standard bacterial media such as blood agar as well as standard fungal media such as Sabouraud's dextrose agar [107]. Certain types of media can be used to increase the sensitivity of culture, with brain heart infusion agar potentially improving the yield [108]. The organism can be cultured from tissue or bodily fluid collected at the site of disease, whether it be a tissue biopsy, blood culture, pulmonary specimen, cerebral spine fluid, or urine. The time to positive culture result for *Cryptococcus* tends to be slower than for bacterial or other yeast organisms, usually 3–5 days before growth is evident [107].

38.2.4.3 Histopathology

Microscopic examination of specimens, either of tissue or fluid, can be an important addition to prompt diagnosis and determining sites of involvement. Several stains can be helpful to distinguish *Cryptococcus* from other fungal infections. India ink has classically been used to highlight the capsule of these yeasts in cerebrospinal fluid. The potential difficulty with this simple test is in successfully identifying the yeast from CSF lymphocytes [107]. With increasing use of easier to perform and interpret antigen tests, however, the India ink is being used less frequently. Gomori methanamine silver (GMS) stain is positive but non-specific for most fungal organisms, and mucicarmine stain of tissue is useful in highlighting the capsule and distinguishing *Cryptococcus* from *Histoplasma, Blastomyces*, and *Candida*.

38.2.4.4 Species Identification

With the increasing recognition of *C. gattii* as a cause of human disease and the comparative difficulties in treating this organism, attention must also be placed on proper species identification with the diagnosis of cryptococcosis. The two main species can be differentiated when grown on agar that is supplemented with L-canavanine, glycine, and

bromthymol blue (CGB agar). *C. gattii* isolates turning the agar blue, while the agar remains yellow with *C. neoformans* isolates [109]. Genetic typing to determine *C. neoformans* or *C. gattii* is generally used with more specialized labs being able to determine the specific subtype of *C. gattii* for epidemiologic purposes and potentially tracing acquisition to a particular exposure [108].

38.2.5 Management

The management of cryptococcosis in SOT recipients is largely based on more recent data generated from the HIV epidemic which has been extrapolated to the SOT population, and abundant retrospective studies specific to post-organ transplantation. Once the diagnosis of cryptococcosis is established, the extent of the infection should be established, in particular to discern whether there is CNS involvement. A lumbar puncture to measure opening pressure and collect cerebrospinal fluid for microbiologic diagnosis is essential in all patients with proven or suspected cryptococcosis. Elevated opening pressure is frequent but not universal. As discussed above, cerebrospinal fluid can be tested for the presence of cryptococcal antigen, stained and microscopically examined for the presence of yeast, and cultured. The presence or absence of involvement in the CNS guides the type and duration of antifungal therapy.

Early studies in HIV patients suggested that amphotericin-B and fluconazole as monotherapy were similar in treating cryptococcal meningitis, however later studies have shown that the combination of amphotericin-B with flucytosine shortens time to sterilization of the CSF and improves outcomes when compared to other interventions [110–112]. Additionally, a prospective study in solid organ transplant recipients noted improved mortality in transplant recipients with cryptococcal meningitis when a lipid formulation of amphotericin was used rather than amphotericin-B deoxycholate [113]. Disseminated disease and fungemic patients also benefit from initial therapy with amphotericin-B, however no specific trials have investigated this. Treatment of isolated, extra-neural disease can be monotherapy with fluconazole, depending on disease severity, with an avoidance of the potential nephrotoxic consequences of amphotericin-B. Similarly, duration of therapy depends on disease location and severity. When amphotericin-B products are being used for induction of disseminated, CNS, or severe disease it should be in conjunction with flucytosine for a minimum of 2 weeks if this regimen can be tolerated [94, 114]. This could potentially be extended if the patient is slow to respond and still with significant symptoms or evidence of disease at 2 weeks. If amphotericin is given without flucytosine, induction should continue for at least 4 weeks [94, 114]. Following the completion of induction therapy, the patient can be transitioned to a fluconazole consolidation phase for 8–10 weeks dosed at 6–12 mg/kg (generally 400–800 mg) daily though dose adjusted, if needed, for renal function [94, 114]. Finally, therapy can be completed with a further 6–12 months of fluconazole maintenance at a lower dose of 200–400 mg daily. For mild to moderate, localized, extra-neural disease that does not require amphotericin-B induction, fluconazole should be given for 6–12 months at 400 mg daily [94, 114].

Other potential options for treatment that have been studied in some fashion are the extended-spectrum triazoles including voriconazole, posaconazole, and isavuconazole, however there are insufficient clinical data to support their use in this setting.

Calcineurin pathways exist in *Cryptococcus* as a means for governing growth and are key in allowing the fungus to grow at higher temperatures, such as that of the human body [115]. Blocking this pathway via the addition of the calcineurin-inhibitors cyclosporine and tacrolimus has been shown to eliminate the ability of *Cryptococcus* to grow at higher temperatures [116]. Indeed, these calcineurin-inhibitors have been shown to act in a synergistic manner with anti-fungal agents against *Cryptococcus* isolates obtained from clinical cases [117]. Additionally, SOT patients with a calcineurin-inhibitor as part of their immunosuppressive regimen appear to have a lower risk of mortality and possibly less CNS involvement in the setting of cryptococcosis compared to those not receiving one of those agents [76].

Another aspect of treatment that should be considered is the potential for drug–drug interactions, in particular with the azole agents. Calcineurin inhibitors require their doses to be decreased when co-administered with azoles [62]. Similarly, mTOR inhibitors also require dose decreases however in an even greater magnitude that can completely restrict their concurrent use [61]. The presence of a an opportunistic infection such as cryptococcosis generally leads to a reduction of the overall immunosuppression as allowed and these interactions require careful monitoring to ensure that goal is met safely.

The European Society for Clinical Microbiology and Infectious Diseases, the Infectious Diseases Society of America, and the American Society for Transplantation have developed recommendations to guide treatment each with specific guidance for SOT patients. These recommendations vary based on the extent of the infection [52, 94, 114].

38.2.6 Complications

One of the hallmarks of CNS cryptococcosis is elevated intracranial pressure. This problem is generally relieved by drainage of fluid via lumbar puncture. If increased pressure persists in spite of drainage, this can lead to a need for more continuous diversion of cerebrospinal fluid. In particular, ventriculoperitoneal shunting has been shown to be safe and effective in managing this issue [118, 119]. A trial to assess the potential of acetazolamide in HIV patients with CNS cryptococcosis to reduce intracranial pressure was terminated early due to serious adverse events and a lack of benefit [120]. There are no studies designed to examine a benefit to steroids for the con-

trol of increased intracranial pressure in the setting of cryptococcal meningitis. One HIV treatment trial did track high dose steroid usage and showed worse mortality and clinical response in those that received steroids compared to those that did not [121]. The Infectious Diseases Society of America guidelines for *Cryptococcus* has specifically recommend against the use of steroids in this setting [114].

With reductions in total immunosuppression in the face of an opportunistic infection, the potential exists to develop immune reconstitution inflammatory syndrome (IRIS). This has been reported in the SOT population [122, 123]. The occurrence of IRIS has been associated with an increased risk of allograft loss in renal transplant recipients [124]. A lack of inflammation in the CSF (fewer than 20 WBCs) at the time of diagnosis has been shown to be a risk factor for the development of IRIS in the HIV population [125]. In the SOT population, discontinuation of calcineurin inhibitors and CNS disease were associated with an increased risk of IRIS, however it did not appear to increase the risk of death [99]. There are no trials to assess potential benefit of steroids or other therapy for IRIS. Anecdotal evidence to support the use of steroids exists and guidelines suggest their use as a component of the treatment of severe IRIS with complications [94, 114, 122].

38.2.7 Mortality

Estimates of mortality at 90 days range from 14% to 21% amongst all SOT patients with cryptococcosis of any type [76, 88, 96]. The TRANSNET study found a 27% mortality at 1 year following infection [1]. Mortality rates appear similar when compared to HIV patients [88, 96].

38.2.8 Prophylaxis

While secondary prophylaxis following cryptococcosis can be considered, there have been no trials to assess for a benefit related to this. Relapse has been reported as rare when patients are appropriately treated [126]. There have been no trials to assess for the potential benefit of primarily prophylaxis in the SOT population.

38.3 Other Yeasts

38.3.1 Trichosporon

Trichosporon is a basidiomycetous yeast found worldwide and in the same family as *Cryptococcus* [127]. The most common species in clinical disease are *T. asahii*, *T. mucoides*, and *T. asteroides* [127]. It has generally been associated with hematologic malignancies, but is reported in a variety of forms in solid organ transplant recipients [128, 129]. Similar to other yeasts as well as bacteria, it can form biofilms on prosthetic surfaces and broadly increase its resistance to anti-fungal agents [130]. The most common forms of invasive disease are fungemia, urinary tract infections, peritonitis, and endocarditis [127]. *Trichosporon* is notable in particular for the poor treatment activity of the echinocandin class of anti-fungal agents [131]. This should be kept in mind in cases of breakthrough yeast infection while patients are being treated with an echinocandin [132]. The activity of the triazoles and amphotericin-B vary according to species, indicating a need to ensure full identification of the organism to allow for optimal treatment [133].

38.3.2 Rhodotorula

Rhodotorula is also a basidiomycetous yeast with a predominance for Asia and the regions of the Pacific [134]. The yeast produces carotenoid pigments and colonies can appear salmon to pink depending on the species isolated [135]. The most common species causing pathogenic disease in humans are *R. mucilaginosa* and *R. glutinis* [136, 137]. The most common form of invasive disease is fungemia, however reports of endocarditis, endophthalmitis, and peritonitis also exist [134, 136, 138]. Reports indicate an association with hematologic malignancies and the presence of a central venous catheter, however it has also been reported in SOT recipients [137–139]. The triazoles have generally poor in vitro activity versus *Rhodotorula* species, but amphotericin-B MICs are generally acceptable [140]. *Rhodotorula* species demonstrate in vitro resistance to the echinocandins, and these agents should be avoided to treat these organisms [138].

38.4 Other Considerations

Dimorphic fungi can appear as yeast forms in tissue specimens and blood cultures. This includes *Histoplasma*, *Blastomyces*, *Coccidioides*, and *Paracoccidioides* as the most common agents. Similarly, patients with fungemia due to one of these organisms are usually initially identified simply as "yeasts," and very often patients are begun on an echinocandin therapy based on an assumption that the organism is a *Candida* species. Echinocandins have little in vitro activity versus the endemic mycoses and *Cryptococcus* species, and should be avoided in these circumstances. Rather, in the proper clinical setting, these organisms should be suspected and thoroughly evaluated through a thoughtful diagnostic work-up and treatment adjusted appropriately. Another potential confounding organism is *Fusarium*, which in spite of being a mold may initially appear as a yeast on blood culture broth, leading to the mistaken impression of a diagnosis of candidemia [141].

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39
Mold Infections After Hematopoietic Stem Cell Transplantation

Kieren A. Marr

39.1 Microbiology and Pathogenesis: An Overview

Pathogenic fungi are typically classified into yeasts, molds, and dimorphics, based on the cellular morphology of the organism that exists outside and inside the body. The most common pathogenic yeasts are within the Basidiomycetes phylum, and these include *Candida* species and *Cryptococcus* species. These organisms exist as unicellular forms in the host and the environment. Some pathogenic fungi switch between a multicellular hyphal and unicellular yeast form, which is a process called dimorphic switching. This switch allows the organism to adapt to different stresses in each environment, with the in vivo switch to yeast typically associated with pathogenicity. This group of phylogenetically diverse organisms within the Ascomycetes phylum includes *Talaromyces marneffei* (previously called *Penicillium marneffei*), *Blastomyces dermatitidis*, *Coccidioides* species (*C. immitis* and *C. posadasii*), *Histoplasma capsulatum*, *Paracoccidioides* species (*P. brasiliensis* and *P. lutzii*), and *Sporothrix schenckii*. They typically cause disease in both immune-competent and immune-compromised hosts, especially in people who have defects in Th1 cellular immunity, which allows latent organisms to escape host control.

This chapter focuses exclusively on infections caused by molds, which are fungi that exist as multicellular hyphal (or filamentous) organisms in both the environment and the host. The most common pathogenic molds fall within the Ascomycetes phylum, with prominent examples including *Aspergillus* species, *Fusarium* species, and *Scedosporium* species. Organisms that are classified within the Mucormycetes phylum have also become more common causes of disease in immune-compromised people; this includes organisms within the genus *Rhizopus*, *Mucor*, *Rhizomucor*, *Absidia*, and *Cunninghamella*. This chapter will focus discussion on three large groups of infections: (1) Aspergillosis, with the term encompassing infections caused by numerous *Aspergillus* species; (2) Mucormycosis, with the term encompassing infections caused by any of the organisms within Mucormycetes; and (3) Other molds, with a focus on the most common "other" infections caused by *Fusarium* and *Scedosporium* species.

This chapter is not meant to serve as microbiology reference, but some understanding of the organisms is necessary in order to have a functional grasp of their diseases, diagnosis, and treatments. Understanding the microbiology of fungi has become complex, in part because identification of these organisms has evolved as scientists have discovered new ways to identify genetic relatedness. As a result, the morphologic identification of key organisms is now known to yield only a rudimentary classification schema. Genetic and multiphasic taxonomy have identified "new" species, or "cryptic" species within many common pathogens. Following the microbiologic classification of pathogenic fungi has become quite complex. For instance, we now know that there are >100 species within the *Fusarium* genus, sometimes with quite a lot of phenotypic variability even within clustered species complexes that are common medical pathogens [1, 2]. Similarly, many of the morphologically identified species within the genus *Aspergillus* are now known to encompass numerous genetically disparate species, some of which have important differences with regard to pathogenicity, growth environments, and susceptibilities to antifungals [3–7]. Clinicians should understand how their clinical microbiology laboratory identifies and reports organisms, as methods vary from conventional morphologic descriptions to DNA sequencing. In this chapter, species names can be assumed to encompass multiple cryptic species, except where clinically relevant differences warrant more discussion.

39.2 Pathogenicity and Clinical Diseases

Aspergillus fumigatus is the most common cause of human disease, and the best studied organism. This organism grows as a mold in the environment, with asexual production of

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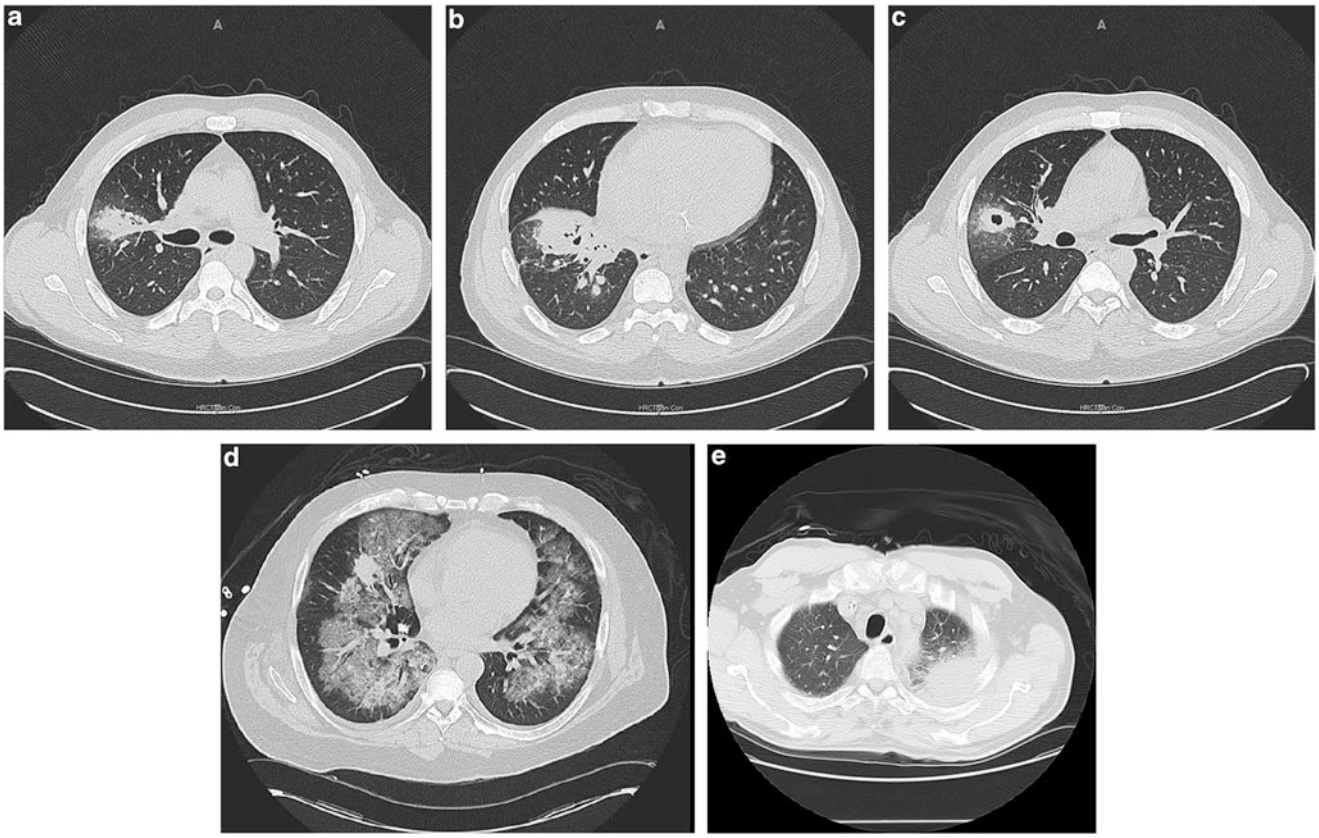

FIGURE 39-1. Radiographic presentations of pulmonary diseases caused by molds. Panels (**a**–**c**) demonstrate progression from a typical nodular infiltrate with halo sign to cavitation within a neutropenic patient during cellular recovery. Panel (**d**) shows a less typical presentation of progressive pulmonary disease in setting of aspergillosis post-engraftment in an allogeneic HSCT recipient. Panel (**e**) shows a bulky infiltrate adjacent to pleura caused by *Rhizopus* spp., which progressed with necrotic inflammation through chest wall.

tiny, hydrophobic conidia (spores) that serve as the primary infective cells into the respiratory tract. In the host, conidia are cleared from the airway, or germinate into filamentous cells, which can trigger destructive inflammatory responses, and/or invade into lung. When secondary immune defenses are deficient—as with neutropenia—these organisms can invade into the blood stream and travel to distant sites, such as skin, brain, and liver. Occasionally, these organisms cause only cutaneous disease, especially after local inoculation into necrotic tissue. More recently, increased awareness has been focused on the potential of *Aspergillus* species, like other molds (e.g., Mucormycetes) to cause local invasive disease in the gastrointestinal tract. This most frequently presents as a complication coincident with multi-organism neutropenic enterocolitis, as focal masses or lesions that cause bleeding, as a source of disseminated infection, or as hepatic abscesses after breach in gut integrity [8–12].

In general, molds are most frequently present with sinus or pulmonary disease. The classic pulmonary presentation is of pulmonary nodules that develop during neutropenia, with symptoms of fever, cough, or pleuritic pain. Radiography can show nodules with a corresponding "halo" that represents focal hemorrhage. These lesions typically increase in size during neutropenia, before ultimately becoming necrotic after neutrophil recovery, seen as a cavity on chest X-ray or CT scan. However, other presentations are more common in non-neutropenic hosts, with development of multifocal infiltrates, tree-in-bud nodules suggestive of tracheobronchial involvement, and even frank airway disease (Figure 39-1).

Other molds typically cause similar syndromes, with a propensity to cause invasive sinus or lung disease. However, there are subtle features that are characteristic of different types of molds. Mucormycetes are typically adept at living within necrotic environments, and pulmonary lesions can sometimes demonstrate a "reverse halo" sign, whereby an inner zone corresponding to tissue necrosis can appear early in the course of pulmonary disease. However, this has been noted with multiple pulmonary diseases other than mucormycoses [13–17]. In addition, these infections may present as large bulky lesions can that progress through tissue planes, boring through pleura, soft tissues, and bone (Figure 39-1).

Some of the other common molds, especially *Fusarium* and *Scedosporium* species, can replicate asexually directly from the filamentous cells in vivo. These molds more frequently

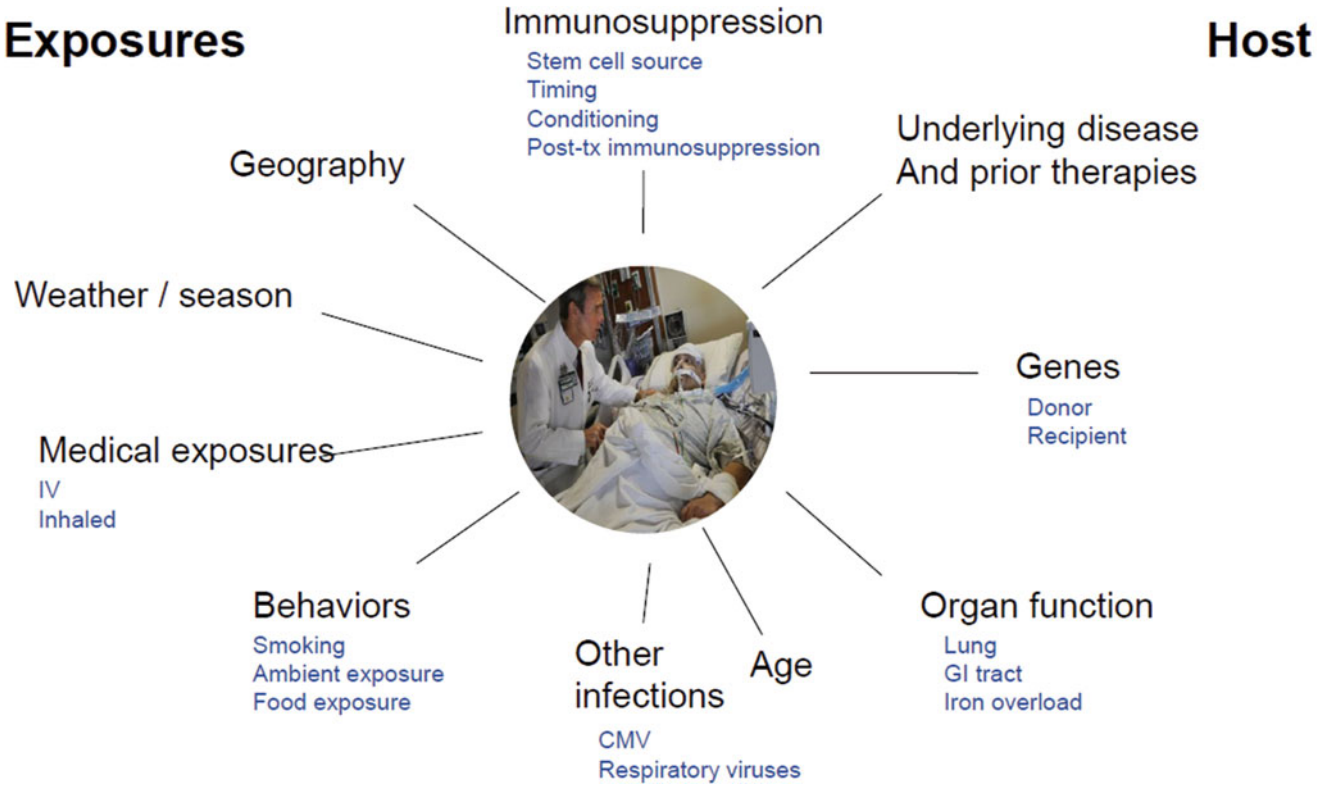

FIGURE 39-2. Schematic diagram of risks that influence probability of developing an invasive mold infection. Multiple variables influence exposure through respiratory and gastrointestinal routes, and host susceptibility to invasive infection [35, 102–108].

grow within the blood stream, and cause disease with higher fungal-burden. Clinically, they may be recognized by growth in blood cultures, and/or with disease that features multiple skin lesions, sometimes in different stages of development. As opposed to *Fusarium* and *Scedosporium*, *Aspergillus* species and Mucormycetes rarely grow from blood cultures, even with documented invasive disease.

39.3 Epidemiology of Invasive Mold Infections

Risks for invasive mold infections (IMI) are summarized schematically in Figure 39-2. Variables that affect exposure to fungi, through both respiratory and gastrointestinal routes, and host susceptibility to invasive disease shape individual susceptibility for these infections. In the sections below, epidemiology will be discussed within the hematopoietic stem cell transplant (HSCT) population specifically.

Invasive pulmonary aspergillosis (IPA) has been known to be a devastating complication of protracted neutropenia for many years. In the 1990s, attention was drawn to high rates of IPA occurring later after HSCT, with multiple single-center studies reporting incidence figures exceeding 10%, with high mortality rates (60–80%) [18, 19]. Today, changes in transplant types (e.g., stem cells and conditioning regimens), diagnostic monitoring, and prevention strategies have largely resulted in stabilization of rates of IPA, as well as improved outcomes after diagnosis [20–26]. Caution is advised in drawing conclusions from different cohort data sets, as these numbers are largely influenced by ascertainment bias given tremendous variability in diagnostic strategies and duration of follow-up across centers. However, one can estimate the incidence of IPA in the range of 5–12% in the highest risk allogeneic HSCT population, and outcomes have improved substantially over time [20–26].

As opposed to IPA, the incidence of infection caused by "other" molds, especially Mucormycetes, *Fusarium* species, and *Scedosporium* species, has increased in the last two decades [27–31]. This may be the result of numerous factors, including diagnostic or reporting bias, given an increased tendency to perform invasive procedures resulting in microbial diagnoses (bronchoscopy), and pressure exerted by use of azoles that have differential activity (e.g., voriconazole). Changes in hosts may lead to increased risks for specific types of infections. For instance, some of the underlying metabolic changes that can occur late after allogeneic HSCT with graft vs. host disease (GVHD)—such as diabetes and iron overload—may specifically predispose to infection with Mucormycetes [32, 33]. In one recent study that utilized data derived from the CIBMTR database, risks for non-*Aspergillus* mold infections (NAMI) amongst a large contemporary

cohort of allogeneic HSCT recipients were assessed [34]. Results suggested that patients with NAMI had more advanced underlying disease and poor Karnofsky performance status at HSCT, with very poor outcomes after infection. Risk factors for mucormycosis include acute GVHD, prior *Aspergillus* infection, and older age. Risks for fusariosis included receipt of cord blood, prior CMV infection, and transplant before May 2002. In this study, infections caused by non-*Aspergillus* molds were diagnosed relatively infrequently, and with similar frequencies in the contemporary antifungal era compared. Risks for infection were largely associated with the severity of illness in the host, and specific factors that predict unusual molds; for example, long-term steroid exposure (even in the HSCT context) is a strong predictor of mucormycosis, while long-term neutropenia is a strong predictor for fusariosis.

When understanding the epidemiology of mold infections, it is always important to consider these organisms as having unique, characteristic environmental growth preferences. For instance, infections caused by *Fusarium* and *Scedosporium* species are most common in the southern USA, Spain, France, and Australia. Weather characteristics can influence risks for aspergillosis and other mold infections, appearing to suggest "outbreak" conditions, but unrelated to single-sources or anthropogenic events (e.g., construction). Examples include increased rates of IPA associated with high ambient spore counts in dry summers in the northwest USA [35] and real outbreaks of specific infections associated with weather disruptions. A recent example of the latter is the development of multiple cutaneous mucormycosis cases that occurred after a destructive tornado in the USA [36]. Understanding the characteristics of these organisms and the underlying endemic natures of exposures assists in developing prevention strategies.

39.4 Diagnostics

Radiology serves as a foundation for early diagnosis of IMI in the setting of suspected disease. As mentioned above, CT scans are especially valuable in providing characteristics of pulmonary disease, and they increase sensitivity of detecting abnormalities compared to conventional chest X-rays. Nodular lesions, halo signs, cavitary lesions, tree-in-bud nodules, focal or multifocal consolidations, and wedge-shaped peripheral lesions are all common presentations of mold complications in the lung. In non-neutropenic HSCT recipients, non-classic abnormalities are more frequently recognized, including more diffuse ground-glass abnormalities and multifocal consolidations [37].

Establishing a microbial diagnosis has become more important than in prior years, as the foundation of optimal drug therapy has leaned away from polyenes, to include azole drugs that have differential activities, and with increased recognition of azole drug resistance amongst common organisms such as *A. fumigatus* [38, 39]. Studies have shown that bronchoscopy, if performed early after recognition of pulmonary abnormalities, has a good sensitivity and is safe [40, 41]. Examination of lavage with culture and augmented testing for fungal antigens (e.g., galactomannan) provide enhanced sensitivity and important information pertaining to likely cause of disease. Biopsy is not routinely employed or necessary, except for cases in which diagnosis is elusive, as risk–benefits shift in patients with different types of lesions and complication risks. In general, biopsy is warranted in patients in whom disease is progressing despite broad antifungal coverage. Yield may be increased with video assisted thoracotomy compared to transbronchial approaches.

Multiple different assays are now commercially available to enhance diagnostic testing for molds, especially *Aspergillus* species. The first, and the most widely employed, is the Platelia serum galactomannan enzyme immunoassay (GM EIA), which utilizes a rat monoclonal antibody in a double-sandwich EIA format. This antibody (EBA2) actually recognizes galactofuranose (galF) side chains of the galactomannan molecule, which is a common constituent of the polysaccharide cell wall of Ascomycetes fungi [42, 43]. The test is now performed in multiple local and regional microbiology laboratories, and although somewhat complex and costly, it provides good information as an aid to diagnose infection with serum or bronchoalveolar lavage (BAL) fluid. Predictive values of test results from BAL are particularly helpful, as in this setting the prevalence of disease is obviously higher than with screening applications on serum [44, 45]. Importantly, detection of "galactomannan" with these methods is not specific for IPA, as multiple organisms, including those that cause similar syndromes (ex. *Fusarium* spp.), express galactomannan—or more specifically—galF antigens that are recognized by the kit antibody [46–48]. Antigens that cause cross-reactivity are also found in multiple drugs, most notably beta-lactam antibiotics [49]. These tests may be falsely positive in HSCT patients, especially in the setting of mucosal breakdown, either early after conditioning or with severe GVHD [50].

More recently, diagnostics that detect other fungal antigens have been developed and cleared by regulatory agencies for use as diagnostic aides. Numerous diagnostic laboratories across the world offer tests that detect beta-D-glucan, largely by harnessing chemical reactions that occur within the limulus amebocyte assay cascade of the horseshoe crab. As this antigen is very abundant on numerous fungal pathogens, and the methods that are employed in these tests serve as a very sensitive primitive immune system of the crab, these tests are typically sensitive, but not specific. Recent studies have reported potential utility of beta-D-glucan tests when used as an aide to diagnose IPA [51, 52].

Numerous centers are now using polymerase change reaction (PCR) tests to assist diagnosis of IMI. Commercial assays and in-house assays have been described and examined in multiple studies. Recent efforts of European groups

have led to optimization of methods to draw and prepare blood products for testing, and for testing itself. These tests also have reasonable performance characteristics, although obviously may vary across tests and labs [53–55].

Finally, new tests developed for use as screening assays and as aides to diagnose IMI are being developed, with a specific focus on aspergillosis. One recent diagnostic cleared for use in the European Union is the lateral flow device that detects a protein antigen in blood or BAL, providing enhanced sensitivity over conventional diagnostics [56, 57]. Others tests in development rely on detection of antigen in unique body fluids (urine) [58] and detection of novel biomarkers, such as fungal metabolites in breath sensors [59]. The landscape of this active field changes daily, limiting over-arching conclusions regarding "best" strategies; however, most would agree that some type of non-culture based method now needs to be employed to optimize diagnostic technologies in centers caring for high-risk HSCT patients.

39.5 Prevention

Efforts to establish preventative strategies have focused on modulating variables that dictate respiratory exposure, enhancing diagnostic screening using radiography or microbial detection, and on employing early antifungal therapy, as part of "prophylactic" or "pre-emptive" strategies. High risks in allogeneic HSCT recipients warrant focus on all of these preventative measures.

Across the world, attention has been drawn to modulating environment in order to minimize airway exposure to inhaled molds. Although centers typically adhere to some standard practice, very little is known about what works best. The high costs and complexities associated with laminar airflow have largely yielded to use of high efficiency air filtration (HEPA) as a standard in housing neutropenic and high-risk transplant recipients within the hospital. Use of masks is typical and widespread, albeit with little data to suggest that it actually works. Results of one small randomized trial evaluating masks failed to show superiority in prevention of documented or suspected IPA, although the trial was small [60].

Serial monitoring of blood using the GM EIA assay and/or *Aspergillus*-specific PCR has been employed in some centers to enhance early diagnosis and to trigger "pre-emptive" therapy, and some randomized trials have evaluated their use. A recent Cochrane meta-analysis that evaluated 18 primary studies encompassing 19 cohorts and 22 data sets reported that both GM EIA and PCR-based screening has moderate diagnostic accuracy when used in high-risk patient groups. This study reported that GM EIA and PCR have good negative predictive value (NPV), useful to exclude disease, but low prevalence limits the ability to rule in a diagnosis. As these biomarkers detect different aspects of disease, some suggest that combinations are likely to be most useful [61]. We lack the large randomized trials necessary to show that we can safely reserve antifungal use in high-risk settings, such as with fever during neutropenia. However, an Australian randomized trial did show that a targeted approach that combined PCR and GM EIA worked to decrease empirical use of antifungals, without observed differences in clinical outcomes amongst 240 patients enrolled [62]. More work—and perhaps better diagnostic tests—will need to be done to fully supplant the widespread use of antifungal therapy for "empiric" indications such as fever during neutropenia, but we may be closing in on that goal.

Importantly, few biomarker-based tests have been developed for non-*Aspergillus* molds, although the non-specificity of beta-D-glucan testing may suggest some utility in future screening strategies. More likely, prevention of these complications will rely on tailored application of prophylaxis, which has also become more widespread after HSCT, largely due to availability of azole drugs that have broad anti-mold coverage. Itraconazole, voriconazole, and posaconazole have all been evaluated in randomized trials for prophylaxis in neutropenic patients and in HSCT recipients, and they are variably used in different centers [63–67]. Early studies that evaluated itraconazole given for prophylaxis in HSCT recipients noted that the drug may be effective at preventing IPA, but with toxicities, mainly associated with the gastrointestinal tract [67, 68]. One large randomized trial reported that posaconazole decreases the incidence of IPA compared to fluconazole in the setting of GVHD, which was consistent with efficacy shown in patients with AML/MDS, leading to its approval for this indication in many countries [63, 69]. Voriconazole has been shown to decrease IPA relative to fluconazole in the HSCT population, although it did not improve the composite outcome of fungal free survival measured late after allogeneic HSCT [65]. This drug has also been associated with decreased mold infections in others at risk populations, including adults and children with neutropenia and steroid receipt [70–72]. Both posaconazole and voriconazole have good, selective activity against different molds, and with variable costs, tolerability, and drug interactions, have been employed at an increasing rate during vulnerable high-risk periods, such as with GVHD.

Amphotericin formulations have also been studied as prophylactic agents in both neutropenic populations and in HSCT recipients. These studies, which have been limited in non-randomized design, or have used alternative dosing regimens of lipid formulations of amphotericin B, have not generated the positive results that have been observed with mold-active azoles, largely due to toxicities [73–75]. Similarly, echinocandins have been studied, and reported to be generally safe when administered as prophylaxis, but these drugs are more effective against *Candida* species compared to invasive mold infections [76]. Finally, positive results have been reported with amphotericin formulations administered via an inhaled route [77, 78] although we don't currently have a commercial product that is easy to administer.

One relatively common situation concerns the need for "secondary prophylaxis" administered to people who have mold infections pre-HSCT. In this situation, a mold-active antifungal should be administered to prevent reactivation of existing infection, or acquisition of a secondary infection. Large observational studies have recorded risks for recurrent IPA in early and late time periods as those that associate inadequate antifungal treatment prior to conditioning, risks that extend durations of neutropenia and those that lead to severe acute GVHD [79, 80]. The best studied drug for secondary prophylaxis is voriconazole, although other products have been shown to work safely in non-randomized trials [81–84].

39.6 Treatment

Table 39-1 presents a summary of key treatment recommendations for the most common mold infections in HSCT recipients. A now "classic" paradigm-changing study showed better survival for people with IPA treated with voriconazole compared to conventional amphotericin B, positioning the mold-active azoles as "first choice" options for therapy [85]. With this in mind, voriconazole is still considered the optimal first-line therapy in multiple consensus guidelines, including those derived by transplant and infectious disease groups. However, amphotericin B formulations, most appropriately the lipid products, which are associated with fewer toxicities, are also reasonable alternatives for treatment of IPA, especially when microbial diagnosis is not confirmed, and in the setting of prohibitive drug interactions or hepatotoxicities. A new azole, isavuconazole, has become available for treatment of IPA as well as other more unusual mold infections, based on a study that suggests non-inferiority compared to voriconazole [86]. Although some early studies demonstrated potential utility of echinocandin monotherapy for mold infections [87], most experts believe that the use of these compounds should be relegated to combination therapy, but not used as a staple.

Combination antifungal therapy has become a topic of increased controversy, with early reports using animal models, case series, and observational comparisons suggesting potentially improved outcomes with different combinations compared to monotherapy. Results of a recent large randomized trial that compared voriconazole monotherapy to combination therapy with anidulafungin suggested trends to improved clinical outcomes, but not definitive superiority of the combination in primary analyses [21]. This study has been variably interpreted worldwide, and run-on studies are being designed to further our understanding of optimal treatment of aspergillosis.

No large randomized trials have been performed to determine the best options to treat the more unusual mold infections, so conclusions are drawn from case series, cohort studies, and small prospective pilot trials. Mucormycoses are typically "best" addressed with lipid formulations of amphotericin B, with aggressive surgical debridement to augment removal of organism and necrosis. These infections can also be treated with some of the newer azole drugs, such as posaconazole and isavuconazole, but no studies have compared outcomes to those of polyenes. Most experts suggest that amphotericin should remain the mainstay primary therapy until more outcomes data are available, and many suggest dose escalations from 5 mg/kg/day to 10 mg/kg/day. Posaconazole and isavuconazole are useful for long-term disease suppression and may have a role for primary therapy, although the latter is not yet well described. Other studies focusing on combination therapies with echinocandin antifungals, iron chelators, and immunomodulators suggest that we may be able to improve outcomes yet further, but more work is necessary to make clear recommendations [88, 89].

Similarly, infections caused by "other molds" that have different antifungal susceptibility profiles have not been well addressed by large treatment studies, but therapeutic regimens are based on anticipated or observed antifungal susceptibilities. Most experts suggest that invasive infections caused by *Fusarium* species be treated with voriconazole as primary therapy, with lipid formulations of amphotericin B as secondary therapy. Others suggest that the combination of both agents may lend to best results, especially in light of antifungal susceptibility differences within the species complexes, but this has not been studied enough to enable widespread promotion by guidelines committees [90, 91]. *Scedosporium* species, especially *S. prolificans*, typically display complex resistance profiles to essentially all antifungal agents, warranting consideration for an aggressive combination approach (Table 39-1).

Detailed discussion of antifungal therapies is beyond the scope of this chapter, but numerous helpful reviews and guidelines have been published [91–93]. An important reminder is that these drugs and organisms are now appreciated to have a more complex susceptibility profile than once believed. Clinical failure of antifungal therapy can represent poor drug levels, especially with mold-active azole drugs, inherent resistance amongst different types of molds, development of drug resistance within a specific organism, and immunologic failure. Not surprisingly, emergence of azole resistance has been increasingly reported, especially in centers located in environments that use azole drugs within agricultural practices [94]. It is likely that drug therapy will be increasingly directed by susceptibility testing in the future, further increasing the need for aggressive diagnostics to establish microbial diagnoses.

Augmented immunologic therapies have been increasingly explored in the HSCT population. Recent efforts have been focused on the utility of granulocyte infusions, and of fungus-specific T cells. One randomized trial that evaluated the safety and efficacy of high dose (GCSF-stimulated) granulocytes halted due to slow accrual, with preliminary reports of safety, but no difference in clinical outcomes in underpowered comparisons [95]. Another study reported that complications were very common when granulocytes were

TABLE 39-1. Summary of treatment recommendations[a]

Infection	Drug	Key recommendations and comments
Aspergillosis		
	Voriconazole	Recommended based on randomized trial results Dose with IV induction followed by oral or IV therapy Doses vary depending on weight and age (more required to maintain appropriate levels in children) Monitoring of levels and adjustment of drug dosing recommended
	Liposomal amphotericin B	Dose 3–5 mg/kg/day
	ABLC	5 mg/kg/day Not studied in randomized trials, diminishing strength of recommendation
	Combination voriconazole + echinocandin	Recommend consideration for early combination therapy in settings of severe infection and in people with severe immunosuppression
	Posaconazole	Used for maintenance in settings with uncertain diagnosis or toxicities with voriconazole
	Isavuconazole	Recommend consideration of this drug in people intolerant to voriconazole given non-inferiority in randomized trial
	Amphotericin B (conventional)	Not recommended for therapy given toxicities and documented poor outcomes compared to alternatives
Mucormycosis		
	Liposomal amphotericin B	Recommended with dose ≥5 mg/kg
	Posaconazole	Recommend consideration of this drug in people intolerant to L-AmB and as maintenance therapy based on case series (no randomized data) Oral extended release tablet preferred over solution formulation given predictability in levels and tolerability
	Isavuconazole	Recommend consideration of this drug in people intolerant to L-AmB given results of case series suggesting efficacy and safety
Fusariosis		
	Voriconazole	Recommended drug given improved outcomes in case series, dosed aggressively as described above
	L-AmB	Recommended in people not tolerant to voriconazole
	Combination therapy	Recommended consideration of combination voriconazole and L-AmB based on predicted risks of drugs and severity of infection
Scedosporium species		
	Voriconazole	Recommended first-line therapy based on case series, dosed aggressively as described above
	Posaconazole Isavuconazole	Case reports and series suggest potential utility but with limited information to date
	L-AmB	Variable activity and outcomes in case series but consideration in people intolerant to azoles
	Combination therapy	Recommended combination voriconazole and L-AmB in severe infection *S. prolificans* with resistance to all antifungals; aggressive combinations of voriconazole and terbinafine should be considered

[a]Recommendations are the author's chosen derivations from numerous guidelines, review of outcomes studies, and experience. References for comments provided in text.

used for treating aspergillosis in the lungs [96]. Some investigators have suggested that granulocyte transfusions may be particularly appropriate in people with disseminated fusariosis; a recent systematic review noted a high proportion of clinical responses after transfusion of donor-stimulated granulocytes, suggesting that this may be an appropriate strategy to "bridge" through neutropenia during active infection [97]. There continues to be great effort directed towards the potential of immunotherapies directed by adoptive transfer of T cells as an adjunct for treating multiple invasive mold infections, with preliminary reports suggesting potential utility as adjunctive therapy for pulmonary aspergillosis [98–101].

Surgery designed to resect focal areas of invasive mold infections is most important in regions where there is a build-up of necrotic tissue, and with imminent vascular compromise. An aggressive surgical approach should be considered with invasive sinus infection, pulmonary masses in approximation to vulnerable vascular structures, and with necrotic lesions associated with mechanical compromise in drug delivery. As mentioned above, surgical resection is most important with infections that are caused by Mucormycetes. Surgery should be approached cautiously with all infections in which there is not as clear a benefit, as complications that involve pleural adhesions and infected empyemas are not infrequent.

39.7 Conclusion

Invasive mold infections have been a frequent, dreaded complication of neutropenia and cellular deficiency, occurring both early and late after allogeneic HSCT. New drugs and diagnostics have resulted in major improvements over the last decades, although these ubiquitous environmental organisms remain a problem during protracted vulnerability. Efforts directed towards enhancing diagnostics and early prevention based on understandings of personal risks, use of highly sensitive screening diagnostics, and tailoring of preventative therapies, are needed to further minimize these complications in the future.

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40 Mold Infections in Solid Organ Transplant Recipients

Patricia Muñoz, Maddalena Giannella, Antonio Vena, and Emilio Bouza

40.1 Epidemiology

40.1.1 The Issue from a General Perspective

Solid organ transplantation has become an important therapeutic option in many diseases. The number of patients undergoing transplantation has increased exponentially in recent years, and the advances in surgical techniques, immunosuppression, and prophylactic strategies have improved allograft and patients' survival [1, 2].

Fungal infections occur in 5–42% of solid organ transplant (SOT) recipients depending on the organ transplanted and the degree of immunosuppression [2–4]. The prevalence of invasive mycosis has declined over the past decade, due in large part to improvements in transplant surgical methods [2, 5]. During this time period, there has been a reduction of infections caused by *Candida* spp. but a rise in infection caused by *Aspergillus* spp. and other less-common mold fungi, such as Zygomycetes, *Scedosporium,* and *Fusarium* spp. [6–9]. The peculiarities encountered with the different types of transplantations deserve some discussion.

The incidence of fungal infections after **heart transplantation** has significantly decreased since the introduction of cyclosporine A (CsA). In the first Stanford series, which was conducted from 1968 to 1978, 35% of the patients had a fungal infection. However, in the past 20 years the incidence was progressively reduced to 10% [10–20] and at present, with the introduction of targeted antifungal prophylaxis, less than 5% of heart transplant recipients will develop an IFI [21–23]. Meanwhile, the incidence of fungal infections, most notably of aspergillosis, after both **lung and heart–lung** transplantation, range from 4% to 10% [1, 24, 25] and are a significant cause of morbidity and mortality.

The incidence of fungal infections in orthotopic **liver** transplant recipients has also decreased over the past 20 years, dropping from between 30 and 50% to 1–9.2% [26–35].

Kidney transplantation currently has the lowest rate of fungal infection [36, 37], with incidence ranging from 0.9% to 4% [35]. Similarly, the outcome of **pancreas** transplantation has continued to improve in recent years, and it is now an accepted treatment for selected patients with type I diabetes, mainly those who have previously undergone kidney transplantation and thus already receive immunosuppressive treatment. Reports of mold infections in these patients are extremely few. In one series on the infectious complications of pancreatic transplantation in 34 consecutive recipients, only one patient developed an infection with *Aspergillus fumigatus* [38].

40.1.2 Onset of Infection

The timing of infections after organ transplantation is quite helpful for suggesting an etiology (Table 40-1) [39–41]. In earlier studies, most cases of mold infections were reported to occur within the first 90 days after transplantation [42, 43]. However, a higher proportion of later occurring cases has been reported in recent series [9, 21, 33, 44, 45]. In the **first month** after transplantation, barrier disruptions and alterations in phagocytic function are dominant, increasing the susceptibility to infections with *Candida*, *Aspergillus,* and the Mucorales [46]. Central nervous system (CNS) infections in SOT recipients during this period are usually due to disseminated aspergillosis [47]. From **1 to 6 months** after transplantation, the type of infection changes, reflecting the shift towards infection caused by pathogens that, in the normal host, are mainly controlled by the cellular immune mechanisms. These include latent infections, such as those caused by the geographically restricted systemic fungi and other mycelial fungal infections [46, 48, 49].

From **6 months after** transplantation and on, approximately 80% of recipients are receiving stable and relatively modest levels of immunosuppressive drugs, and the incidence of fungal infections clearly decreases in these individuals [48]. The 10–20% of patients who never do well and who continue to take large amounts of immunosuppressant drugs or who develop a posttransplant neoplasia, may, however, experience aspergillosis and other opportunistic mold

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Table 40-1. Timing of fungal infections in SOT recipients

Early (<1 month)
Uncommon except in liver transplant recipients
Nosocomial intensive care unit *Candida* infections
Central nervous system abscess (most *Aspergillus*)
Pulmonary *Aspergillus* (aspiration/ventilation)
Intermediate (1–6 months, especially 3 months)
Candida
Aspergillus
Zygomycoses (Mucorales)
Other molds
Late pathogens
Aspergillus
Cryptococcus
Endemic fungi
Sporothrix schenkii
Dematiaceous fungi

Modified from Patterson JE. Epidemiology of fungal infections in solid organ transplant patients. *Transpl Infect Dis.* 1999;1:229–236, with permission.

infections late after transplantation [33, 46, 48, 50]. Seronegative CMV recipients who receive prolonged antiviral prophylaxis may develop CMV infection late after transplantation also increasing their susceptibility to opportunistic agents such as molds [51].

40.1.3 General Risk Factors

The basic risk factors for fungal infection are qualitatively similar in all SOT recipients [52] and the incidence varies with the following two factors: the intensity of exposure and the patient's susceptibility to infection. With regards to the latter, factors contributing to fungal infections include immune defects created by the underlying disease that led to the need for transplantation in the first place [43, 53–57], the type of organ transplanted, the occurrence of technical or surgical complications [29, 43, 53, 55, 57–61], the presence of neutropenia, thrombocytopenia, or metabolic alterations (e.g., diabetes), the intensity of immunosuppression, viral infections, the need for additional antirejection therapy, and renal failure and hemodialysis among others [29, 33, 57, 61–68].

40.1.4 Morbidity and Attributable Mortality

Fungal infections are a major cause of morbidity and mortality [3]. This is due in part to the difficulty of establishing the diagnosis early so that the infection is not disseminated at the time of diagnosis [46, 69], as well as to the limited efficacy and toxicity of some of the available antifungals [70].

The mortality associated with invasive fungal infections (IFIs) varies with the type of fungus, the organ transplanted, and the location of the disease. In heart transplant recipients, the overall mortality rate of fungal infection ranges from 29% to 36% [15, 17, 19, 21] with a marked decrease in most recent studies. In lung transplant recipients, the mortality rate of bronchial infections, which is roughly 20%, is lower than that of invasive pulmonary mycoses, which ranges from 67% to 82% [1, 71–73]. In liver transplant recipients, the mortality rate has ranged from 83% to 88% [74–76]. More recent studies have reported better outcomes with mortality ranging from 10% to 33.3% [1, 77]. Among kidney transplant recipients, patients with an IFI have a relative risk of mortality of 2.88 (95% CI = 2.22–3.74) compared with those who do not have IFI [5].

40.2 Infections Caused by *Aspergillus* spp.

40.2.1 Incidence

SOT patients constitute the third group in frequency among patients with Invasive Aspergillosis (IA), after patients with hematological malignancies and chronic pulmonary diseases [78]. As already mentioned, the incidence of IA varies according to the type of SOT from less than 2% to more than 25% [30, 42, 61, 70, 79–86].

In **heart** transplantation, *Aspergillus* is the predominant fungal isolate (70% of IFIs), accounting for 38% of all nodular lesions observed in the lungs of heart transplant recipients [29, 70, 74, 81, 82, 87–92]. The incidence of IA in this population ranges from 3.3% to 14% (average 6%) [15, 67, 92–94]. In both lung and heart–lung transplantation, colonization with *Aspergillus* occurs at some time after transplantation in 25–30% of **lung** transplant recipients [95–97]. IA occurs in 3–15% (average of 6%) of the patients [92, 95, 97–100]; 58% of these infections are tracheobronchitis or bronchial anastomotic infections, 32% are invasive pulmonary aspergillosis, and 22% are disseminated infections with extrapulmonary involvement [100]. Compared with heart, lung, or heart–lung transplantation, *Aspergillus* infection after **liver** transplantation is less common (1–8% of recipients) [43, 53, 55, 75, 83]. Although *Candida* species are the primary cause of fungal infection in these patients [31, 37, 101], *Aspergillus* can account for as many as 25% of all fungal infections in liver transplantation [35, 102]. In **kidney** transplantation, the cumulative incidence of *Aspergillus* infection is usually less than 5% (from ~0.7% to 4%) [29, 70, 90, 91], a figure which is similar to that observed in pancreas transplant recipients [86, 103].

Mortality associated with IA is nearly 100% if the disease is not treated [104]. The attributable mortality of IA varies depending on the type of infection, is still higher than 70% for disseminated disease with CNS involvement but has significantly decreased for pulmonary IA [21, 33, 77, 105, 106].

40.2.2 Microbiology of the Genus *Aspergillus*

Aspergillus is one of the most ubiquitous airborne saprophytic fungi. In both immunocompetent hosts and in SOT recipients, the species of *Aspergillus* that most commonly causes invasive disease is *A. fumigatus.* Far less common are those infections caused by *A. flavus*, *A. niger,* and *A. terreus* [20, 95, 107–109]. Notably, the first case of human infection by *Aspergillus granulosus* has been reported in a cardiac transplant patient [110]. New molecular methods have allowed the detection of less-common species (cryptic species) that frequently can be resistant to most commonly used antifungals. Resistance to voriconazole has also been described in *A. fumigatus* species, so the judicious use of antifungals is now more important than ever [111–114].

40.2.3 Onset of Infection

In earlier studies, most cases of IA were reported to occur within the first 100 days after transplantation (mean time was 17d for LT, 45 for HT, 82 for KT, and 120 for Lung T). However, in recent years a significant percentage of cases occur later. This has led to the differentiation of IA into early-onset infection, occurring <90 days after transplantation, and late-onset infection, occurring after that period. Such bimodal pattern is related to different risk factors. In fact, *Aspergillus* infection occuring in the first moths after transplantation is likely a sign of an unusually intense environmental exposure [40].

In a large retrospective study on 11,014 SOT recipients from Spain, IA occurred a mean of 234 days (range, 2–3025 days) after transplantation [33]. Early IA accounted for 57% of the episodes and 43% were late. Risk factors for early IA include a complicated postoperative course, repeated bacterial infections or CMV disease, and renal failure or need of hemodialysis. Late IA developed more commonly in older patients with an over-immunosuppressed state due to chronic graft rejection or allograft dysfunction and in patients with posttransplantation renal failure or neoplasia.

In **lung** transplant recipients, *Aspergillus* infection occurs a median of 3.2 months after transplantation, with 51% occurring in the first 3 months after transplantation and 72% within 6 months [100]. Tracheobronchitis or anastomotic infections tend to occur early, while invasive pulmonary and disseminated infections usually appear later after transplantation.

In **heart** transplant recipients, the usual time of IA onset is 36–52 days posttransplantation, with nearly 75% of the cases occurring within the first 90 days after transplantation. At one institution, the onset of IA was significantly delayed after the introduction of routine ganciclovir prophylaxis [94]. We have recently published a 24-year experience with IA in HT recipients. IA was diagnosed in 6.5% of heart transplant patients, with a significant decrease in recent years. Incidence decreased from 8.7% (24 of 277) in the period 1988 to 2000 (historical cohort) to 3.5% (7 of 202) afterward (p 0.02); four of the seven recent cases were in the context of an outbreak. In our study, IA occurring during the first 90 days after transplantation (23/31 cases, 75%) presented most commonly with lung infection, whereas episodes occurring later (8/31, 25%) showed a higher frequency of disseminated disease including CNS involvement. Related mortality was 36% with a significant reduction during recent years (46% vs 0%; p 0.04) and a trend toward lower related death in early vs late cases (26% vs 63%, p 0.09) [21].

Historically in **liver** transplant recipients IA was an early disease (16–17 days after transplantation) [59], but the onset is now delayed. A study showed that the percentage of IA occurring after 90 days of liver transplantation increased from 23% in 1990–1995 to 55% in the period 1998–2002 [44]. Improved outcome in the early postoperative period, and delayed onset of posttransplant risk factors such as CMV infection, allograft dysfunction due to recurrent hepatitis C virus hepatitis are proposed as potential explanations [44].

40.2.4 Pathogenesis and Risk Factors for Aspergillosis

This ubiquitous organism may colonize normal or immunocompromised hosts by spore inhalation. Some strains are more pathogenic than others as a study conducted in the author's hospital demonstrated; in this study, the elastase activity of the *Aspergillus* strains correlated with the invasiveness of the strain [115].

The respiratory tract is the portal of entry in as many as 95% of cases of IA [48]. Once tissue infection develops, invasion of blood vessels is the rule, accounting for the three cardinal features of IA: tissue infarction, bleeding, and dissemination with metastatic seeding [91].

The following two epidemiologic patterns are observed: a domiciliary pattern, in which the exposure occurs in the room or ward where the patient is housed, and a nondomiciliary pattern, in which the exposure occurs as the patient travels within the hospital for a diagnostic or therapeutic procedure [48]. The discovery of domiciliary outbreaks is usually relatively easy because of clustering of cases in time and space [116]. At present, this risk is attenuated largely because of better technical management of the endotracheal tube and the use of high-efficiency particulate air (HEPA) filtration [76]. Clustering is not usually present with the nondomiciliary exposure, and this type of infection usually requires a significant amount of exposure [48, 117].

Skin colonization may also occur and primary cutaneous aspergillosis has been associated with occlusive dressings [118–120]. *Aspergillus* spp. may also invade the gastrointestinal tract or, rarely, they may gain entry through an intravenous catheter [90]. The transmission of the infection via the

donor graft has also been described [121–123]. Donor-derived mold infections have been mostly described in cases in which the donor had CNS manifestations or was himself an immunosuppressed patient.

The host risk factors for aspergillosis are similar to those associated with other invasive mold infections and have been previously commented [68, 106]. Alveolar macrophages normally kill inhaled conidia, and functioning neutrophils eliminate residual mycelia. Therefore, both neutropenia and the dysfunction of macrophages and neutrophils secondary to steroid administration in SOT recipients predispose the recipient to the infection. A body of evidence now suggests that T-cell function and adaptive immunity characterized by a dysregulated production of T-helper (Th) cell cytokines play a pivotal role in the pathogenesis of IA [124–126]. Indeed, Th1 responses have been shown to confer protection against *Aspergillus*, while Th2 responses have been associated with disease progression [124, 125]. Both calcineurin-inhibitors and corticosteroids, administered to SOT recipients to prevent allograft rejection, produce a down-regulation of Th1 responses [127].

Signal transduction mediated by Toll-like receptors (TLRs) has also been shown to play a key role in immunity against aspergillosis [128, 129]. *Aspergillus* conidia stimulate both TLR2 and TLR4 to induce a Th1 cytokine response. Germination of hyphae leads to the loss of TLR4-mediated signals with an ultimately predominant Th2 response. An association between the donor TLR4 haplotype S4 and the risk of IA among recipients of hematopoietic-cell transplants from unrelated donors has been shown [129]. Recently the presence of Pentraxin 3 (PTX3) polymorphisms have been shown to increase the risk of IA in SOT recipients [130].

In SOT patients who require prolonged intubation and ventilatory support, who develop renal failure and need hemodialysis, or who present CMV infections, the risk of IA is further increased [40, 48]. Renal failure and hemodialysis have been shown to impair T-cell proliferative responses and result in an increase in activation-induced T-cell death [68, 106, 131]. A heightened susceptibility to IA among transplant recipients with CMV infection is believed to result primarily from cell-mediated immunosuppressive effects of the virus [63]. CMV may also affect the respiratory burst of macrophages.

40.2.5 Clinical Manifestations of *Aspergillus* Infection in Different Transplants

In a retrospective multicenter study of 11,014 SOT recipients conducted in Spain by the *Spanish Network for Research on Infection in Transplantation* (RESITRA), pulmonary infections accounted for 59% of the IA cases, comprising 20.5% nodular cases and 38.4% pneumonia cases. Disseminated aspergillosis was diagnosed in 41% of IA, with CNS involvement in 15.4% of cases [33].

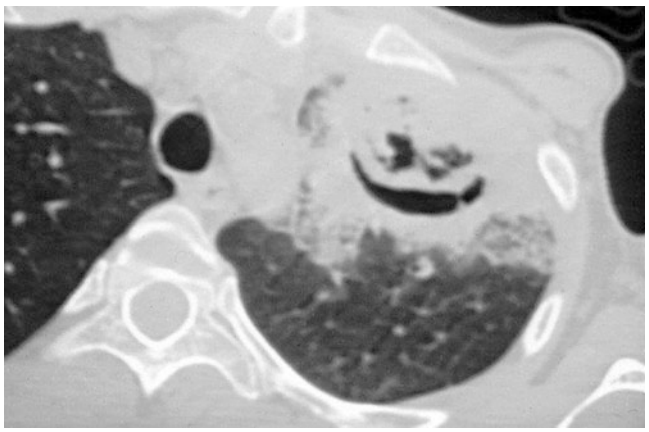

FIGURE 40-1. Pulmonary aspergillosis in a heart transplant patient.

Although pulmonary aspergillosis frequently presents with single or multiple pulmonary nodules with or without cavitation (angio-invasive aspergillosis) [12, 15, 82, 93] (Figure 40-1), a new radiological manifestation (airway-invasive aspergillosis) characterized, according to CT findings, by peribronchial consolidation or a tree in-bud pattern should also be considered. In our series of 27 heart transplant patients, 37.1% of the cases presented with this airway invasive disease. These patients had more need of hemodialysis after transplantation, more intercurrent bacterial pneumonia, and the infection was diagnosed later after symptoms onset (2.7 vs 8.5 d, p 0.09). After diagnosis, AIR-IPA patients required more mechanical ventilation (23.5% vs 90%, p 0.01) and had a higher related mortality rate (23.5% vs 70%, p 0.04) [132].

Aspergillus may disseminate from the lungs to almost any organ [133–138]. Overall, disseminated disease has been described in up to 20% of cases with higher percentage among liver recipients [106]. Disseminated infection with CNS involvement occurred in 17% of the cases studied in Spain [82]. Brain abscesses are relatively uncommon (0.6%) in SOT patients; however, 78% of those observed are caused by *Aspergillus* species [139]. Clinical manifestations of CNS aspergillosis include an alteration of mental status, diffuse CNS depression, seizures, evolving cerebrovascular accidents, and headache [76, 85, 140]. The CSF fluid is usually sterile.

Recently, Kourkoumpetis et al. reported 14 cases of CNS aspergillosis and reviewed 123 cases reported in the literature between 2000 and 2011. Solid organ transplantation was the underlying disease in 8.9% of the cases and in most patients CNS involvement resulted from dissemination from the paranasal sinuses or lungs. Of note, a significant proportion of patients had a history of previous brain pathology, which could predispose them to metastasis of infection to the CNS [141]. Of particular interest is the fact that CNS infection caused by fungi is often associated with extraneural infection with the same microorganism, which allows the sampling and evaluation of an established infection without a need to perform a brain biopsy. In a series on CNS lesions in liver transplant recipients, all of the brain abscesses found were fungal

and *Aspergillus* organisms were the most frequent isolate (75%). Furthermore, 73% of the patients had concurrent extraneural (pulmonary) infection that was caused by the same fungal pathogen [142]. Therefore, the consensus is that a diagnostic brain biopsy is not warranted in SOT recipients with CNS lesions. Occasionally, however, the colonization of extraneural sites may confuse diagnosis [46].

One study revealed that *Aspergillus* organisms were a common cause of infective endocarditis (26%) that occurred within a month of solid organ transplantation [143]. Other forms of clinical presentation include mediastinitis and osteomyelitis [10, 15]. Skin and soft-tissue infections also occur [144, 145]. In heart transplant recipients, cardiac aspergillosis probably originated in the atrial suture or in the surgical site has been reported [146].

In **lung and heart–lung** transplant recipients, disease presentation can also include bronchial anastomosis dehiscence, vascular anastomosis erosion, bronchitis, tracheobronchitis, invasive lung disease, aspergilloma, empyema, disseminated disease, endobronchial stent obstruction, and mucoid bronchial impaction [52]. Kramer et al. described a distinct form of IA after lung transplantation, in which ulcerative tracheobronchitis, a semi-invasive disease involving the anastomosis site and the large airways, was encountered [147]. Bronchial stent obstruction is particularly problematic because 15% of patients will show some degree of airway narrowing, bronchomalacia, or airway stenosis [52]. The placement of an artificial stent may be then required; however, the artificial stent seems to be a trap for fungi, leading to fungal tracheobronchitis, impaction, and pneumonia. These stents have to be cleared often and mucous plugs containing *Aspergillus* are found within these stenosed airways.

In **liver** transplant recipients, aspergillosis of the wound occasionally occurs [59, 148, 149]. Other clinical manifestations are primary cutaneous infection after trauma [119], the infection of a biloma [150], gastrointestinal dissemination and systemic involvement with endocarditis [143, 151, 152] or endophthalmitis [153–155]. CNS involvement is particularly more likely to occur in liver recipients who have been retransplanted. However, disseminated and CNS infections have declined in the current era [44, 156]. It has been proposed that the in vitro anti *Aspergillus* activity of calcineurin and target-of-rapamycin inhibitor agents may have a protective effect on the risk of disseminated aspergillosis [157].

As in the other transplant groups, pulmonary aspergillosis is the most frequent clinical presentation of aspergillosis in **kidney** transplant recipients [36, 85, 108, 158–166]. Other clinical presentations include cutaneous aspergillosis [118, 167], genitourinary disease [168–174], arthritis [175, 176], isolated cerebral aspergilloma [177], epidural abscess and paraparesis [178, 179] and invasive or disseminated disease [136–138, 166, 180–187]. A potentially highly lethal form of invasive aspergillosis in kidney transplant recipients is pseudoaneurysm of the iliac artery that may be transmitted by a graft contaminated during the preservation phase [188].

40.2.6 Diagnosis of Invasive Aspergillosis

The diagnosis of IA in SOT recipients remains a significant challenge, since 25–33% of these patients may be asymptomatic initially or they may have nonspecific symptoms. Furthermore the progression of the disease is fast. The diagnosis is also crucial, mainly because the prompt recognition of these infections, combined with intensive antifungal therapy, is needed for cure [3, 74, 90, 189, 190]. The diagnosis is reached by a combination of different methods, including clinical findings, isolation of the microorganism, radiology, the serologic detection of antibodies or antigens and histopathologic evidence of invasion [191, 192].

40.2.6.1 Clinical Findings

Clinical manifestations can be subtle and may include fever and pulmonary findings, such as chronic cough or chest pain that result from angioinvasion of fungi; these may be important clues to the diagnosis of infection [90, 132, 192] and in high-risk patients may be enough to establish a presumptive diagnosis of infection and to allow institution of therapy pending a confirmation of the diagnosis [74].

40.2.6.2 Imaging Techniques

Chest radiographic findings in patients with pulmonary *Aspergillus* include nodular opacities, interstitial infiltrates, cavitary lung disease, or a pulmonary embolus pattern; however, the chest X-ray may also be normal [81, 139, 193]. In patients with infection due to angioinvasive molds, especially those with invasive pulmonary aspergillosis, computed tomography (CT) scanning is particularly valuable in diagnosing the disease when the chest radiograph remains negative or shows only minimal changes. In SOT recipients, high-resolution CT scanning of the chest is more sensitive than plain radiographs, and it may reveal disease as much as 5 days earlier [139]. In these patients, a pulmonary halo sign is less likely to correlate with IA, as it may occur with other types of pneumonia as well [132, 189]. In heart transplant recipients, the chest CT provided significant additional information in 41% of the patients with IA (mainly greater spread of the disease); and, more importantly, only the chest CT showed abnormalities (normal chest X-ray) in 18% of the cases [21].

Since mold infections in this population primarily affect the lungs and sinuses, diagnostic approaches should concentrate on these two anatomical locations, with the early and repeated, if needed, performance of CT scans. In patients with brain lesions, the use of CT scans or magnetic resonance imaging of the neuroaxis is essential for the assessment of the presence and nature of infectious processes. To date, the utility of positron emission tomography with F18-fluorodeoxyglucose (FDG-PET) to diagnose IA in SOT recipients is not determined [194]; data suggesting the utility

of FDG-PET in the diagnosis of invasive aspergillosis is mainly extrapolated from studies including hematological patients.

40.2.6.3 Etiologic Diagnosis

The currently available laboratory diagnostic methods include histopathologic evidence of invasion, isolation of the microorganism or demonstration of the DNA by molecular methods and the serologic detection of antigens or antibodies. The newer techniques remain mainly investigational at this time.

40.2.6.4 Histopathologic Evidence of Invasion

Aspergillus hyphae are 2–4 mm wide, and they frequently septate and branch at 45°. They are best-visualized with silver stains, and may be missed when the only routine hematoxylin-eosin stains are applied. In rapidly progressive infections, the hyphae are of even diameter, whereas in more chronic cases they may have bulbous, widened areas. Sporulation is rarely observed in the tissue, except for in areas containing air. In the absence of sporulation, *Aspergillus* hyphae cannot be readily distinguished from a large number of pathogenic molds, and the mycologic differential diagnosis includes *Scedosporium*, *Fusarium*, *Scopulariopsis,* and many other rarer molds.

Definitive proof of IA requires the culture of *Aspergillus* from a sterile site (e.g., brain aspirate or an abscess). Histologic evidence of hyphal invasion of the tissue with concurrent positive culture of *Aspergillus* from the same site (e.g., a transbronchial biopsy showing hyphae and a positive sputum culture for *Aspergillus*) is also definitive evidence of the disease [195]. Angiotropism and angioinvasion are important histologic characteristics of invasive mold infections. However, other fungi (e.g., *Fusarium*, *Zygomycetes)* may invade tissue and produce similar histologic images.

40.2.6.5 Isolation of the Microorganism

Pathogenic species of *Aspergillus* usually grow easily and quickly on the routine bacteriologic and mycologic media used in the clinical laboratory. The identification is relatively straightforward by microscopic criteria and the colony (conidial) color. Formal identification may require the performance of cultures on specialized media such as Czapek-Dox and malt extract. Species identification is warranted in transplant units. As mentioned, *A. fumigatus* is the most common species recovered from cases of invasive aspergillosis, followed by *A. flavus*, *A. niger*, and *A. terreus* [20, 95, 107–109]. At present molecular methods are very helpful to identify isolates at species level [196].

Increasing evidence indicates that positive cultures for *A. fumigatus* should not be overlooked in the appropriate epidemiologic and clinical setting (e.g., highly immunosuppressed transplant recipients) because this is strongly associated with the existence or risk of IA [42, 87, 197]. However, the early diagnosis of IA in SOT recipients is difficult because fungal cultures in these patients may be negative even when infection is widely disseminated [74, 90]. For instance, blood cultures are notoriously insensitive for *Aspergillus* infections, even those producing endocarditis [3]. Furthermore, the confirmation of the diagnosis may require obtaining tissue for culture using an invasive procedure [198], which may sometimes not be viable because of the increased risk involved. In HT recipients the diagnosis of IA possible only after obtaining an invasive sample (bronchoalveolar lavage or biopsy/aspiration) in 29% of the patients [21].

In IA, the sensitivity of positive respiratory samples from SOT recipients for *Aspergillus* differs depending on the type of transplant [44, 97, 199]. In a multicenter Spanish study in **heart** transplant recipients, the sensitivity of various respiratory tract cultures for the diagnosis of IA were as follows: sputum examination, 45%; bronchoalveolar lavage (BAL), 83%; bronchial washing, 100%; transbronchial biopsy, 50%; open-lung biopsy, 50%; and transthoracic needle aspiration, 50% [19]. During a 10-year study period, *Aspergillus* spp. were recovered from 10.5% of heart transplant recipients and a positive predictive value (PPV) of 60–70% for IPA [197]. When analyzed by species, the positive predictive value of recovering *A. fumigatus* was 78–91%, whereas it was 0% for other species. The positive predictive value increased from 88% to 100% when *Aspergillus* was recovered from a respiratory specimen other than sputum and decreased from 50% to 67% when it was recovered only from sputum [197]. The performance of BAL is most valuable in patients with diffuse changes on CT scans [3].

In a multicenter Italian study of IFI in **lung** transplant recipients, the use of BAL for the diagnosis of IA had a sensitivity of 100%; a specificity of 92%; and positive and negative predictive values of 16 and 100% [92]. *Aspergillus* spp. can be detected in airway samples from ~25% to 30% of lung transplant recipients [95, 100]. Although airway colonization with *Aspergillus* organisms correlates with a low positive predictive value for IA in lung transplant recipients, it is associated with a higher risk of subsequent invasive infection [37, 189, 200]. The risk of progression from colonization to IA is increased in lung transplant recipients with lower values of T-cells response (<50 ng/mL ATP with the Cylex ImmuKnow assay) [201]. Patients with positive airway cultures for *Aspergillus* within 6 months after transplantation were 11-fold more likely to develop IA [202] and should undergo a bronchoscopic examination to exclude the presence of tracheobronchitis or invasive disease. In another report, the isolation of *Aspergillus* organisms from a respiratory specimen was associated with a 22-fold greater risk for subsequent IA.

In **liver** transplant recipients with invasive pulmonary aspergillosis, *Aspergillus* species are detected in respiratory

secretions (sputum or BAL) in 50% of these individuals [56]. Colonization of the respiratory tract with *Aspergillus* organisms is uncommon in liver transplant recipients (~1.5%), but it has a high positive predictive value of subsequent development of IA, ranging from 41% to 72% [189].

The diagnosis of extrapulmonary infection is usually more difficult [3, 203]. Any suspicious lesion (e.g. skeletal or cutaneous) should be biopsied and cultured for fungi according to the recommendations found in the current guidelines [3, 195, 203].

Although the Clinical Laboratory Standards Institute has developed a standardized method for mold susceptibility, a clinical correlation of increased minimal inhibitory concentration for molds has not yet been established with regard to the susceptibility testing of fungal isolates [111, 204]. Azole resistance by *Aspergillus* species is increasingly reported in recent years [112, 113], and some patients exposed chronically to antifungal triazoles have been reported to have refractory infection caused by isolates with elevated MICs [205, 206]. The clinician must realize that some molds are going to be less susceptible to some classes of antifungal drugs (e.g., *A. terreus* is clinically resistant to amphotericin) [1]. Nevertheless, predicting the expected susceptibility pattern of a particular mold is much more difficult that it is for yeasts. Because of the emerging development of resistance, testing the susceptibility of *Aspergillus* organisms and other molds to the available antifungal drugs is warranted in these infections.

40.2.6.6 Serology

In recent years great advances have been performed in the serologic diagnosis of IA, mainly in hematological patients, allowing a rapid diagnosis. The serologic diagnosis of IA is based on the detection of circulating antigens in biologic fluids, such as serum, urine, or BAL fluid [207, 208]. Briefly, the Platelia (Sanofi Diagnostic Pasterur, Marnes la Coquette, France) sandwich-enzyme immunosorbent assay (ELISA) for the detection of galactomannan (GM) is currently one of the more used methods. A recent meta-analysis showed a greater utility of the GM test in neutropenic bone marrow transplant recipients than in SOT patients, in whom sensitivity of serum samples was 71% and specificity 89% [209]. In a retrospective study of IA in liver transplant recipients, Fortun et al. found a sensitivity of GM of 55.6% and a specificity of 93.9%, with a positive predictive value of 71.4% [210]. GM was not very useful either in heart transplant recipients [21]. In heart transplant recipients GM showed a sensitivity of 28.6% [21]. Other specimens, such as bronchoalveolar lavage (BAL), have been proven to be more advantageous in this population. At the index cutoff value of ≥1, the test yielded sensitivities and specificities ranging from 60% and 98%, respectively in lung transplant recipients and 100 and 91% in liver transplant patients [200].

Although in hematological patients GM test may enable the early diagnosis of IA [211], and monitor treatment response [212, 213], further studies are mandatory in SOT patients since these aspects have not been specifically proven in this population.

Unfortunately, false negative and positive results are not uncommon in SOT recipients. False negative results of the GM assay have been related to the previous use of antifungal agents (44% of lung transplant recipients with false negative GM) [1]. False positive GM results are common in lung (20%) and liver transplant recipients (9–13%), mainly in the early postransplantation period [24]. Potential causes are the specific underlying conditions leading to the transplantation (e.g., cystic fibrosis and chronic obstructive pulmonary disease, autoimmune liver disease and dialysis requirement), or administration of betalactams [1].

Other tests, such as the detection of cell wall markers like the galactosaminoglycan or **1-3-β-** d **-glucan**, or the polymerase chain reaction (PCR) are still not so widely used. 1-3-β-D-glucan is a cell wall component of yeasts and molds [214]. It is detectable in blood during IFI caused by the *Aspergillus*, *Fusarium,* and *Acremonium* species [214]. The test proved to be useful for the diagnosis of invasive aspergillosis in living-donor liver allograft recipients in one study [215]. Some authors have proposed that a reasonable approach to the clinical application of these assays is serial screening (weekly or biweekly) of patients with a high risk of IFI. Once a positive result is obtained and confirmed, the use of an assay with high fungal specificity (e.g., GM sandwich-ELISA for aspergillosis) could help to narrow down the specific type of fungal infection further and could aid in the choice of targeted preemptive antifungal therapy [216].

Recently, the diagnostic value for IFI of the 1-3-β-D-glucan was questioned in the transplant population because of the low positive predictive value of the test. Indeed, in a study performing 1-3-β-D-glucan on BAL and serum samples from 135 SOT patients with proven, probable, or no IFI, the sensitivity, specificity, positive and negative predictive values of the test were 79.2, 38.5, 27.6, and 86.3% in BALs and 79.2, 81.8, 69.2, and 83.1% in serum sample tested [217].

The use of **PCR** to detect invasive fungal pathogens, including *Aspergillus*, is receiving increasing attention. PCR screening for *Aspergillus* has a sensitivity, specificity, and negative predictive value of 100%, 65% and 100%, respectively [218]. Some protocols use universal fungal PCR primers that enable the detection of a broad range of fungi [219–221], and a sensitivity of 1–10 fg of fungal DNA can be achieved. In one study, the sensitivity of the PCR used on whole blood was 100% in patients with documented IFI when two or more samples were analyzed [220]. In one study in renal transplant recipients blood panfungal PCR preceded clinical signs of invasive fungal infections by 27 days [222]. Quantitative PCR can be used to monitor the fungal level to indicate the response to treatment, similar to the use of PCR monitoring in cytomegalovirus disease [223].

The results are better when the technique is performed on bronchoalveolar lavage specimens, with sensitivities and specificities of pan *Aspergillus* PCR reaching 100% (95% CI 79–100%) and 88% (95% CI 79–92%), respectively.

However, PCR-based molecular diagnostic tests for *Aspergillus* are not commercially available, remain largely unstandardized, and their precise role in the diagnosis and management of invasive aspergillosis in SOT recipients remains to be determined [224]. Finally, it should be noted that the specificity of PCR performed on lower respiratory tract samples is moderate, as a positive result only indicates the presence of *Aspergillus*, and does not allow distinguishing between colonization and infection [225].

Recently, a **lateral flow device** (LFD), detecting a glycoprotein antigen found in the serum and BAL of patients with IA [226], has been proposed as a new diagnostic approach for detecting IPA in immunocompromised populations, including SOT patients [227]. An early semiprospective multicenter study evaluating LFD device in BAL from SOT patients (26 lung transplants, 13 liver, 6 kidney, and 2 heart transplantation) showed a sensitivity, specificity, positive and negative predictive values for probable IA of 91%, 83%, 63%, 97%, respectively [228]. However, despite initial promising results, further and larger studies are warranted before safe conclusions on the performance of *Aspergillus* spp. LDF can be reached. Clinical trials evaluating the role of *Aspergillus* LFD as an alternative to GM in serum and BAL fluid are currently underway (clinicaltrial.gov identifier NCT 02058316).

Finally, different technologies detecting volatile organic compounds exhaled in the breath of patients infected with IA have been recently tested [229–231] with increasing enthusiasm by the scientific community. However, their role in SOT patients remains to be clarified.

40.2.6.7 Differential Diagnosis

The clinical presentations of fungal infections in SOT are nonspecific, and they often overlap with other infectious and noninfectious processes. Therefore, a histologic and microbiologic analysis is mandatory. In any fungal infection diagnosed in a SOT patient, a careful search should be made for metastatic infection, especially if the location at which the infection was found involves the skin, the skeletal system, and/or the CNS. The clinician must remain aware of this type of infection because, in many cases, the diagnosis is only established postmortem.

40.2.7 Treatment

Early treatment with an effective antifungal drug decreases the high fatality rate associated with these infections. The development of new antifungals and the performance of antifungal susceptibility tests have enabled longer survival in patients who contract fungal infections [232]. However, the emergence of multidrug-resistant pathogens, medication toxicity and drug–drug interactions must be carefully evaluated [233].

The drugs used in the treatment of deep fungal infections in SOT recipients do not differ significantly from those used in other types of immunocompromised hosts, although antifungal treatment in this specific population has special features [90]. The therapy is complicated by the following three factors: (a) the continuing need for immunosuppression, except for recipients of kidney and pancreas allograft recipients whose condition may be maintained with dialysis and insulin therapy if the graft fails; (b) the need for a prolonged course of therapy; and (c) the potential for all of the currently available antifungal agents to interact with essential immunosuppressive drugs, particularly CsA [46, 91, 234–241].

40.2.7.1 Antifungal Agents

Over the past decade, there has been a considerable research in antifungal drugs targeted against IA [106, 195]. To date, the antifungal agents licensed for the first-line treatment of IA include amphotericin B and its lipid formulations, itraconazole, voriconazole, posaconazole, and caspofungin [195]. Micafungin and anidulafungin, which are also candins, have in vitro, in vivo, and clinical activity against *Aspergillus* but are not yet licensed for primary therapy of IA [195]. The severity of the infection, the clinical form, renal insufficiency, the drug interactions, the availability of the drug, and its cost are some of the factors that can help in making the selection of the best drug.

Voriconazole is a triazole, for which both oral and intravenous formulations are available. It has a broad spectrum of antifungal activity that includes *Candida* species, *Aspergillus* species, *Cryptococcus neoformans*, fungi that cause the endemic mycoses, *Fusarium* species, *Paecilomyces lilacinus*, *Scedosporium apiospermum,* and dematiaceous fungi [242–246]. It is not active against Zygomycetes, however. It has good penetration of both the CSF and brain.

The largest randomized trial for primary therapy of invasive pulmonary aspergillosis demonstrated that voriconazole was superior to amphotericin B deoxycholate, followed by other licensed antifungal therapy [247]. This study included 11 SOT recipients. At week 12, successful outcomes were observed in 52.8% of the patients in the voriconazole group and in 31.6% of those in the amphotericin B group. The survival rate at 12 weeks was 70.8% in the voriconazole group and 57.9% in the amphotericin B group (hazard ratio, 0.59; 95% CI 0.40–0.88). Patients treated with voriconazole had significantly fewer adverse events that were drug-related, except for transient visual disturbances. Therefore, the authors of this study concluded that voriconazole was more beneficial for the treatment of IA than amphotericin B.

Other series studying SOT recipients, with proven or probable invasive pulmonary aspergillosis, confirmed a

favorable response rate with voriconazole [232, 248, 249]. Particularly remarkable is one study of CNS aspergillosis, including 25 SOT recipients, treated with voriconazole for a median of 93 days (range 1–1128), that showed a favorable response rate of 48% (40% for SOT) [250]. Intravitreal voriconazole has also been used in a lung transplant patient with *Aspergillus* endophtlamitis [251, 252]. Because of better survival and improved response of initial therapy with voriconazole, this agent is now considered the drug of choice for primary therapy of IA in most patients, including SOT recipients, by the recent Clinical Practice Guidelines of the Infectious Diseases Society of America (IDSA) (A-I level recommendation) [195].

Voriconazole is generally well tolerated but early recognition of side effects may be extremely important. Patients taking voriconazole can develop skin rash, prolongation of QT interval, visual disturbances, and hepatic enzyme elevation [253, 254]. Periostitis, skeletal fluorosis, and exostoses have also been reported [255, 256] mainly, but not always, in patients receiving long-term therapy. Clinical presentation includes multifocal pain in different skeletal regions with elevation of alkaline phosphatase and of plasma fluoride level [257, 258]. Bone scintigraphy reveals areas of increased radiotracer uptake and prompt discontinuation of voriconazole should result in improvement of symptoms. Early recognition of this phenomenon is important to prevent unnecessary tests and procedures. In a recent report a consistent number of patients reported nail changes and alopecia associated with prolonged voriconazole therapy [259]. Rare cases of cutaneous squamous cell carcinoma [260–265] have also been described.

One issue of paramount importance in the treatment of SOT recipients with IA is the drug interaction of antifungal agents with the immunosuppressant agents. The triazole agents are potent inhibitors of the CYP34A isoenzymes and have the potential to increase the levels of calcineurin-inhibitor agents and sirolimus [266]. Itraconazole has been shown to increase CsA or tacrolimus levels by 40–83% [267] and a 50–60% reduction in the dose of calcineurin-inhibitor agents may be necessary with the concurrent use of voriconazole [266]. The use of sirolimus is contraindicated in patients receiving voriconazole, although some authors have safely coadministered them with sirolimus dose reduction by 75–90% [268–272].

Coadministration of posaconazole increased cyclosporine exposure and necessitated dosage reductions of 14–20% for cyclosporine [273]. Posaconazole increased the maximum blood concentration and the area under the concentration-time curve for tacrolimus by 121% and 357% respectively [273].

For patients who are intolerant of or refractory to voriconazole, a **lipid formulation of amphotericin B** is regarded as an appropriate alternative. Beginning in the early 1990s and for almost a decade, lipid formulations of amphotericin B, have been the mainstay of the treatment for IA in SOT recipients, largely because of a lower potential of nephrotoxicity than conventional amphotericin, but probably also due to less efficacy. A study compared the efficacy of amphotericin B lipid complex (median dose of 5.2 mg/kg/day) and amphotericin B deoxycholate (median does of 1.1 mg/kg/day) for the treatment of IA in SOT recipients [274]. The overall and IA-related mortality rate was 33 and 25% in the amphotericin B lipid complex group, and 83 and 76% in the amphotericin B deoxycholate group [274]. In another study of 47 SOT patients with IA who were treated with lipid formulations of amphotericin B (5–7.4 mg/kg/day), the overall 90-day mortality was 49% and the IA associated mortality was 43% [275]. Based on the AmBiLoad study, in which liposomal amphotericin B at the dose of 3 mg/kg/d of per day showed similar efficacy to 10 mg/kg/d and less toxicity, doses higher than 5 mg/kg/d are not recommended [276]. Some clinicians initiate therapy with conventional amphotericin B and switch to a lipid formulation only with patients who cannot tolerate the drug or who are refractory to treatment. However, in SOT recipients, the use of conventional amphotericin B in the high dose required for the treatment of IA is associated with significant renal toxicity, mainly in patients receiving CsA or tacrolimus [57, 277]. Accordingly, if available, the authors of this chapter recommend starting therapy with one of the lipid-based formulations. If infusion related symptoms appear, such as fever, chills, headache, nausea, and vomiting, the use of preparative medication for subsequent infusions is appropriate. Aerosolized amphotericin B deoxycholate or lipid formulations of amphotericin B have been used for the treatment of tracheobronchial infection in SOT, although this approach has not been standardized and remains investigational [195].

Caspofungin is the only candin currently approved by the US FDA for the first line therapy of IA. It inhibits glucan synthesis of the fungal cell wall. At this time, this is only available in an intravenous formulation. It is highly active against *Candida* and *Aspergillus* infection, but comparative data for amphotericin or voriconazole in SOT patients are not available. In a study that employed caspofungin as primary therapy for IA in 12 SOT recipients, the response rate was 92% [278]. Although caspofungin has been used successfully as salvage therapy in IA as single agent [279] and in combination with other drugs [280], a recent study analyzing 181 hematological patients with IA reported an higher *Aspergillus*-mortality rate in patients treated with primary salvage therapy with caspofungin compared with voriconazole alone. In the same study, the combination of voriconazole and caspofungin did not result in a better outcome [281]. The pharmacokinetics of caspofungin is unaltered by coadministration of tacrolimus, but caspofungin may reduce tacrolimus concentrations by up to 20% and may increase cyclosporine A plasma concentrations by 35% [282]. Caspofungin should be used with caution in patients treated with CsA due to elevated liver function tests [87, 282, 283].

To date limited experience exists with the use of **posaconazole, micafungin, or anidulafungin** for the treatment of IA in SOT recipients [284]. Anidulafungin showed no clinically

relevant drug interactions with immunosuppressors used in SOT [285, 286] and micafungin a mild inhibitor of cyclosporine levels and increased sirolimus serum concentrations by 21% when administered concomitantly [287].

40.2.7.2 Combination Therapy and Other Considerations

The high lethality of IA justifies any attempt to improve monotherapy by combination therapy. As yet, no conclusive results are available and updated guidelines of the IDSA suggest reserving this option for salvage therapy [195]. However, several authors have shown that the combination of amphotericin B, caspofungin, and voriconazole generally has a synergistic or additive effect in vitro and in experimental models [288–300].

A prospective, multicenter study in SOT recipients compared outcome in 40 with IA patients who received primary therapy with voriconazole plus caspofungin with 47 patients, from an earlier cohort, who received a lipid formulation of amphotericin B [275]. The two groups were well matched, including the proportion with disseminated disease (10% vs. 12.8%), proven IA (55% vs. 51.1%), or *A. fumigatus* (71.1% vs. 80.9%). Overall survival at 90 days was 67.5% in the cases and 51% in the control group. Mortality was attributable to IA in 26% of the cases and in 43% of the controls ($p=0.11$). Deaths tended to occur later in cases than in the control patients (mean 49.5 vs. 36.7 days, $P<0.11$). In the multivariate Cox regression model, CMV infection and renal failure were independently predictive of mortality at 90 days. Combination therapy was associated with a trend towards lower mortality (HR 0.58, 95% CI: 0.30–1.14, $p=0.117$) when controlled for CMV infection and renal failure. When 90-day mortality was analyzed in subgroups of patients, combination therapy was independently associated with reduced mortality in patients with renal failure, and in those with *A. fumigatus* infection, even when adjusted for other factors predictive of mortality in the study population [275]. No correlation was found between in vitro antifungal interactions and outcome. None of the patients required discontinuation of antifungal therapy for intolerance or adverse effects however, patients in the combination therapy arm were more likely to develop an increase in calcineurin-inhibitor agent level, or gastrointestinal intolerance [275].

A retrospective survey of invasive pulmonary aspergillosis in lung transplant recipients (9/19 disseminated), showed a mortality rate of 86% (12/14) in patients who received Amphotericin B preparations (amphotericin B deoxycholate or a lipid formulation of amphotericin B) and 0% (0/3) in those who received voriconazole plus caspofungin [301]. Mortality rate in patients receiving voriconazole plus caspofungin was also lower compared to lipid formulations of amphotericin B (0/3 vs. 8/8, $p=0.006$).

A higher response rate and superior survival was also observed in a retrospective series of CNS aspergillosis when antifungal combination therapy was administered [250].

The most important data supporting the role of combination therapy for treatment of IA have been recently provided from a randomized, double-blind, placebo-controlled multicenter trial comparing voriconazole and anidulafungin with voriconazole monotherapy [302] The main results of this study are described in this chapter.

In many transplant programs in Europe combination therapy constitutes the standard antifungal therapy for invasive aspergillosis in SOT recipients [303, 304]. Surveys of antifungal therapeutic practices for IA in transplant recipients documented that, currently, combination therapy is used as first-line treatment in 34–47% of the transplant centers, and as salvage therapy in 58–80% of them [305, 306].

However, not all antifungal combinations are beneficial, and some may be deleterious [195]. Pending definite clinical results and considering cost, treatment with the combination of voriconazole and caspofungin or anidulafungin may be less costly than therapy with 5 mg/kg/day of liposomal amphotericin B [307].

Therapy should continue after the resolution of disease and of the reversible, underlying conditions that originally predisposed the patient to the development of the IA. The optimal duration of therapy is unknown; it is dependent on the extent of IA, the response to therapy, and the patient's underlying disease(s) or immune status. A reasonable course would be to continue therapy for the treatment of microfoci until after the clinical and radiographic abnormalities have resolved; any cultures, if they are readily obtainable, are negative; and any predisposing factors have abated. The IDSA guidelines recommend a minimum of 6–12 weeks in normal hosts and maintenance throughout the period of immunosuppression and until lesions have resolved in immunosuppressed patients [195]. Long-term therapy of IA is facilitated by the availability of oral voriconazole in stable patients. The clinical response, rather than an arbitrary total dose, should guide the duration of therapy.

Therapeutic monitoring of invasive pulmonary aspergillosis includes serial clinical evaluation of all symptoms and signs, as well as performance of radiographic imaging, usually with CT, at regular intervals. The frequency of CT should be individualized on the basis of the rapidity of the evolution of pulmonary infiltrates and the situation of the individual patient. The use of serial serum galactomannan assays for therapeutic monitoring is promising but remains investigational in SOT [305]. Progressive increase in *Aspergillus* antigen levels over time implies a poor prognosis; however, resolution of galactomannan antigenemia to a normal level is not sufficient as a sole criterion for discontinuation of antifungal therapy [195].

A growing body of evidence suggests patient-to-patient variability in the pharmacokinetics of triazoles [308, 309].

Absorption issues (for itraconazole and posaconazole), drug–drug interactions (for all triazoles), and pharmacokinetic differences (for voriconazole) all contribute in various degrees to this variability [195]. Although the available data are still scarce, accumulating reports suggest that plasma drug levels monitoring may play an important role in optimizing the safety and efficacy of azole antifungals [309]. In a study of 12 lung transplant recipients, a few patients were identified to have nearly undetectable concentrations midway or throughout the entire dosage interval [310]. On the other hand, it has been demonstrated that patients with voriconazole levels ≥5.5 mg/L where more likely to develop neurological toxicity [309]. Accordingly, until more data are available, the authors recommend systematic monitoring of voriconazole and posaconazole levels.

40.2.7.3 Additional Treatments

Surgical excision or debridement remains an integral part of the management of mold infections as it has both diagnostic and therapeutic purposes [85, 134, 144, 203, 311]. Surgery is specifically indicated if hemoptysis, particularly a life-threatening hemoptysis, is present; if lesions are impinging on the great vessels or major airways, because of the higher likelihood of catastrophic pulmonary hemorrhage; if the infection is sinonasal infections; if a single cavitated lung lesion is present that increases despite adequate treatment; if the pericardium, bone, or subcutaneous thoracic tissue is infiltrated while the patient is receiving antifungal drugs; and when intracranial abscesses are present [195]. A pneumonectomy was successfully performed for progressive, refractory angioinvasive aspergillosis in a lung transplant recipient whose disease progressed despite the use of conventional antifungal therapy [312].

Adjunctive therapy has been used in some situations and in vitro and animal studies has shown enhanced antifungal activity and modulation of cellular immune responses when cytokine or colony stimulating factors are provided [313–315]. However, no definitive role for the use of cytokines as adjunctive treatment to antifungal drugs has been established. Granulocyte and granulocyte-macrophage colony-stimulating factors and interferon-γ (IFN-γ) all increase both phagocytosis and the damage to *Aspergillus* hyphae in vitro [316]. However, no clinical benefit has been demonstrated either in the phase I and II studies of macrophage colony-stimulating factor or in a retrospective series of cases of IA in which granulocyte-colony-stimulating factor was used [317]. Guidelines of the IDSA suggest a role for IFN-γ as adjunctive antifungal therapy for invasive aspergillosis in immunocompromised non neutropenic host [195]. The use of this cytokine in organ transplant recipients is of concern, however, given the risk of potential graft rejection.

The ultimate response of these patients to antifungal therapy is largely related to host factors, such as a decrease in the immunosuppression and the return of graft function from a transplanted organ, as well as the extent of the aspergillosis when it is diagnosed. Reducing immunosuppression by withdrawing corticosteroids and decreasing the doses of CsA and tacrolimus is an important adjuvant measure to surgical treatment. In these cases, close monitoring of CsA and tacrolimus concentrations is of paramount importance. Graft function should also be followed closely in those recipients whose graft is essential for survival.

The treatment of *Aspergillus* species infection in SOT recipients is summarized in Table 40-2.

40.2.8 Prevention of Invasive Aspergillosis

Antifungal prophylaxis for SOT recipients remains a complex and controversial issue mainly due to the scarcity of large clinical trials. Antifungal prophylaxis may be provided to the whole population (universal) or only to high-risk patients (targeted). Both strategies are effective and have not been sufficiently compared, so definite recommendations cannot be given and each center will have to choose the best strategy depending on their epidemiology, experience, and access to diagnostic and therapeutic tools. However, targeted prophylaxis is the preferred option in most transplantation programs, considering the risk of toxicity and emergence of resistant isolates and that risk factors for IA are well defined, are only present in a minority of SOT recipients and during short periods of time [68, 106].

40.2.8.1 Prophylaxis in Lung Transplant Recipients

Lung transplant recipients are at high risk of suffering different types of *Aspergillus* infections, so the need of prophylaxis should be considered in every patient. However, different indications, drugs, and length of therapy are used in different programs and no clear superiority has been demonstrated by any specific strategy [318]. Some centers still use universal prophylaxis while others only treat patients with risk factors.

Preemptive antifungal therapy may also be used in lung transplant recipients and in some liver transplant recipients when *Aspergillus* colonization is detected with the aim of treating the pathogen before it causes disease in high-risk patients [73, 106]. This strategy is mainly used when pulmonary colonization by *Aspergillus* species is found immediately before or within 6–9 months after transplantation [86, 91, 319, 320]. Not enough data exist on prophylactic strategies based on positive galactomannan in serum or bronchoalveolar lavage in lung transplant recipients.

TABLE 40-2. Treatment of infections by *Aspergillus* species

	Treatment	Duration
Invasive disease[a]	**Primary therapy** Voriconazole (6 mg/kg iv every 12 h for 2 doses, followed by 4 mg/kg iv every 12 h; oral dosage is 200 mg every 12 h) **Alternative** Liposomal amphotericin B (3–5 mg/kg/day iv) or Amphotericin B lipid complex (5 mg/kg/day iv) or Caspofungin (70 mg day 1 iv and 50 mg/day thereafter) Micafungin (iv100–150 mg/day; dose not established) or Posaconazole (200 mg QID initially, then 400 mg BID orally after stabilization of disease) Itraconazole (dosage depending upon formulation)	See text
Tracheobronchial infection	**Primary therapy** Voriconazole (6 mg/kg iv every 12 h for 2 doses, followed by 4 mg/kg every 12 h; oral dosage is 200 mg every 12 h) **Alternative** Liposomal amphotericin B (3–5 mg/kg/day iv) or Amphotericin B lipid complex (5 mg/kg/day iv) or Itraconazole (dosage depends upon formulation) with or without inhaled nebulized amphotericin B (6 mg/kg q8h) or Caspofungin (70 mg day 1 iv and 50 mg/day thereafter) or Micafungin (iv 100–150 mg/day; dose not established) or Posaconazole (200 mg QID initially, then 400 mg BID orally after stabilization of diseased)	At least 21 days or until the patient is asymptomatic and cultures are negative
Colonization	Inhaled nebulized amphotericin B 6 mg/8 h Voriconazole 200 mg/12 h orally Itraconazole (dosage depend upon formulation)	At least 15–21 days or until cultures become negative
Ulcerative tracheobronchitis[b]	Voriconazole (6 mg/kg iv every 12 h for 2 doses, followed by 4 mg/kg every 12 h; oral dosage is 200 mg every 12 h) or Liposomal amphotericin B (3–5 mg/kg/day iv) or Amphotericin B lipid complex (5 mg/kg/day iv) or Itraconazole (dosage depends upon formulation) + Inhaled nebulized amphotericin B (6 mg/kg q8h)	Clearance of fiberoptic signs and negative cultures

[a]Combination therapy (voriconazole + caspofungin) is suggested in all cases of IA as primary therapy.
[b]Surgical debridement or excision of mycelial masses is necessary.

Regarding the drug of choice nebulized amphotericin B, with or without an azole, is probably the most frequently used option. In a recent study, including 27 of 64 (45.5%) active LTx centers in the United States, antifungal prophylaxis increased from 52.3% in 2011 to 77.8% in 2013, with the most common agent being inhaled amphotericin B (61.9%), followed by oral voriconazole (51.9%). A total of 74.1% of centers treat *Aspergillus* airway colonization, with 80.0% of centers using oral voriconazole. All centers treat IA, with 92.6% using oral voriconazole.

The incidence of IA in lung transplant recipients has been shown to decrease with nebulized amphotericin B (0.2 mg/kg every 8 h) [81, 321–325]. Nebulized liposomal amphotericin B appears to be more effective than conventional amphotericin [325, 326].

In one study oral itraconazole with or without nebulized liposomal amphotericin B effectively prevented invasive disease in colonized patients [327]. However, some cases of semi-invasive tracheobronchial aspergillosis were detected that required higher serum levels (more than 1000 mg/L) are required for cure [327].

Another study compared universal prophylaxis with voriconazole to preemptive prophylaxis with itraconazole, with or without inhaled amphotericin B, for the prevention of IA in lung transplant recipients [73]. The study suggested the superiority of universal prophylaxis over the targeted strategy, since all but one patient who developed IA in the itraconazole group had not received the drug. The use of voriconazole was, however, associated with abnormal liver enzymes in a significant percentage of patients [73]. Considering the possibility of adverse events and high risk of drug–drug interactions, the role of newer triazoles as antifungal prophylaxis in lung transplant recipients remains to be fully defined. Voriconazole is, however, preferred by some authors when the reason for starting the prophylaxis is the colonization of the respiratory tract with *Aspergillus* or *Scedosporium*. Prophylaxis is not indicated when other molds are recovered due to the significantly lower risk of infection.

The duration of prophylaxis is not clearly established and varies widely across centers from 3 months to >1 year in the US. Some center adjust the duration of prophylaxis to the type of patient [326]. In uncomplicated lung transplant recipients, prophylaxis is generally administered for approximately 2 weeks after surgery. In complicated lung transplant recipients, nebulized amphotericin B is administered for 4 weeks during the period of greatest immunosuppression after IA has

been ruled out. In patients who experience a rejection episode, a course of prophylaxis may be administered during the period of greatest immunosuppression; in those with repeated episodes of rejection or chronic rejection, prophylaxis may be maintained on a continuing basis. Other authors recommend the administration of nebulized amphotericin B for several weeks after transplantation or until the bronchial anastomosis has healed [86]. Others maintain that this should be lifelong therapy (6 mg/day) [325, 326].

40.2.8.2 Prophylaxis in Other Solid Organ Transplant Recipients

At present, the prophylaxis of infections by molds is not universally recommended for SOT recipients. Routine antifungal prophylaxis should be aimed at high-risk patients.

Usually antifungal prophylaxis is not recommended in **kidney transplant** recipients.

Universal antifungal prophylaxis is not warranted **in heart transplant** recipients. However, in patients deemed to be at high risk, itraconazole (400 mg daily from day 5 after transplantation to month 3 or 6) was associated with a significantly lower incidence of invasive aspergillosis and lower overall mortality (2% versus 9.6%; $P<0.05$) [67]. In a more recent study, we analyzed the security of adapting the indication and duration of antifungal therapy to the specific risk factors present in each patient, instead of using a fixed and empirical prophylaxis [22]. We performed a prospective study in which prophylaxis was only administered to heart transplant recipients with risk factors for invasive aspergillosis (13 of 133 transplanted patients; 9.8%). The duration was personalized, starting with the risk factor and continued 15–20 days after its resolution. Antifungal prophylaxis consisted mainly on the administration of candins and was effective in all but one patient who should have received a higher dose of caspofungin due to his obesity. Despite suffering an outbreak of invasive aspergillosis (IA) in the intensive care unit due to extremely high concentration of spores in the air (three cases with no personal risk factors), there was a reduction in the incidence of IA (8.6% vs. 2.2%; $P=0.01$) and *Aspergillus*-related mortality (5.75% vs. 1.5%; $P=0.06$) [22].

This study also demonstrated that a high environmental load of *Aspergillus* spores in the intensive care unit would also indicate the need for antifungal prophylaxis in all exposed transplant recipients [116].

A meta-analysis of antifungal prophylactic trials **in liver transplant** recipients documented a beneficial effect on morbidity and attributable mortality, but an emergence of infections due to *non-albicans Candida* [328]. Since the risk factors and the period of susceptibility to invasive fungal infections is clearly definable, antifungal prophylaxis targeted towards these high-risk patients is deemed to be the most rational approach in this population. A lipid formulation of amphotericin B—at a dose of at least 3 mg/kg/day—or caspofungin have been used [248, 329–331]. High-risk patients should be targeted, and the prophylaxis should continue for a period of 4 weeks after transplantation or while the risk factors remain present [151, 248, 329–332].

Most authors recommend preemptive therapy for patients who have acute, **fulminant liver failure** before transplantation, since they are considered high-risk recipients [319]. This approach advocates initiating the therapy on admission to the intensive care unit and continuing it after transplantation for 3–4 weeks, based on the concept that *Aspergillus* infection is diagnosed early after transplantation in these patients [59].

A recent prospective, multicenter, noncomparative, open-label trial performed in Spain by GESITRA evaluated the efficacy of the preemptive administration of caspofungin in **adult liver transplant** recipients at high risk of developing Aspergillosis [283]. A total of 71 patients were enrolled in the study and received caspofungin for at least 21 days. Two patients (2.8%) developed an invasive fungal infection—a *Mucor* and a *Candida albicans* surgical wound—respectively, after caspofungin therapy was finished (41 and 19 days, respectively). Six more patients (8.4%) discontinued caspofungin because of drug-related altered liver function. Hence, successful treatment outcome was obtained in 88.7%. None of these patients developed IA. The authors conclude that caspofungin is an efficacious and well-tolerated drug as antifungal prophylaxis in high risk liver transplant recipients [283].

Anidulafungin was safe in a retrospective series of adult solid organ transplantation (SOT) recipients [333]. Sixty-two patients (72%) received anidulafungin for prophylaxis. There was no need for the modification of immunosuppressive drug doses and no patient discontinued anidulafungin because of severe adverse effects. While receiving anidulafungin, one patient developed mild liver toxicity, but the liver function normalized without the discontinuation of anidulafungin.

A recent prospective, open-label, noninferiority, multicentric study (TENPIN study) analyzed the role of preemptive antifungal therapy with micafugin in high risk liver transplant recipients (48.0% had a Model for End-Stage Liver Disease score ≥ 20). Patients were randomized to receive intravenous micafungin 100 mg or center-specific standard care posttransplant. Overall, 344 patients were included: 172 micafungin; 62 fluconazole/59 L-AmB/21 caspofungin. Both arms of prophylaxis were very effective (98.6% for micafungin and 99.3% for standard care). At end of prophylaxis there were 12 episodes of invasive fungal disease, 8 candidiasis and 4 aspergillosis (2 in the micafungin arm and 2 in the standard of care—1 fluconazole and 1 L-AMB). Tolerance was excellent in both arms, although kidney function was better with micafungin. Adverse events leading to drug discontinuation was 6.4% for micafungin and 11.6% in the standard of care. At EOP, liver function tests were similar but creatinine clearance was higher in micafungin- vs standard care–treated patients [334].

TABLE 40-3. Recommended fungal prophylaxis against mold infections for solid organ transplant recipients

Type of transplant	Fungal pathogen targeted	High-risk factors	Antifungal agents	Duration of prophylaxis
Liver	*Aspergillus*[a]	Tx for fulminant hepatic failure Retransplantation Reoperation involving thoracic or intraabdominal cavity Post-Tx hemodialysis CMV infection	Echinocandins or Lipidic formulations of amphotericin B	3–4 weeks
Heart	*Aspergillus*	Post-Tx hemodialysis CMV disease Reoperation High concentration (>25 cfu/m3) of *Aspergillus* spores in the ICU or other case of IA in the Tx program	Echinocandins or itraconazole or voriconazole	During the risk factor and 2 more weeks
Lung	*Aspergillus*	Airway sample culture positive for *Aspergillus* CMV infection Obliterative bronchiolitis Rejection Increased immunosuppression	Nebulized liposomal amphotericin B ± Echinocandins Voriconazole, or Itraconazole	4–6 months ± Prolonged or perhaps indefinite

Modified from [106] and [68].
Abbreviations: *CMV* cytomegalovirus infection; *OKT3* monoclonal anti-T-cell antibodies.
[a]Liver transplant recipients with airway sample cultures positive for *Aspergillus* should be considered for therapy for invasive aspergillosis.

Another clinical trial, also including liver transplant recipients with risk factors for IFI, compared anidulafungin and fluconazole administered during a median of 21 days after transplantation [335]. This randomized, double-blind trial showed that the incidence of IFI (5.1% anidulafungin and 8.0% fluconazole) was similar in both arms. There were two cases of IA in the fluconazole arm, but not a single patient died of IFI. The results of this study are provocative, as the risk factors for Candida and Aspergillus frequently overlap and both microorganims have to be prevented in many patients. A summary of the previously commented are summarized in Table 40-3.

40.2.8.3 Avoidance of Epidemiologic Exposures After Transplantation

a) Hospital exposures. The Centers for Disease control and Prevention (CDC) guidelines suggest that hospital policies should minimize the exposure of high-risk patients to potential sources of molds (The CDC specifically refers to *Aspergillus* pathogens in neutropenic patients). These should be put into practice, especially during situations such as hospital construction and renovation. The routine use of HEPA filters in the wards is not required, with the exception of those wards containing neutropenic patients. HEPA-filtered air-handling systems should be used when the potential for the contamination of the air supply exists [336]. In addition, SOT recipients should wear special masks when they are being transported through high-risk areas within the hospital.

   In our experience, the detection of >25 cfu/m3 in nonprotected hospital air, such as an ICU without HEPA filters, is abnormal. The efficacy of antifungal drugs in these high-risk situations (i.e., in hospitals with a high incidence of fungal infections, high concentration of spores or during an outbreak) is not well established. However, when a significant nosocomial environmental exposure is demonstrated and considering the specially high susceptibility of solid organ transplant recipients [67, 116], we recommend the administration of antifungal prophylaxis to all transplants admitted in the affected area [21, 22].

   In addition, flowers and potted plants, which may be colonized with *Aspergillus*, should not be permitted in patient rooms. This restriction must also be applied to food products (e.g., nuts, cereals, spices) that may be colonized with mold spores.

b) Community exposures. A particular association has been noted between invasive pulmonary aspergillosis and gardening; therefore this hobby should be discouraged in SOT recipients [48].

40.2.8.4 Microbiological Surveillance

Microbiologic screening for fungal infections before and after SOT can be conducted for the donor graft an in the recipient [337].

a) Donor graft

   Fiberoptic bronchoscopy with microbiological sampling should be performed routinely in the lung donor in the case of lung or heart–lung transplantation [338].

b) Recipient

In the case of single lung transplantation, the remaining native lung may harbor serious opportunistic infec-

tions. Examining the excised lung of the recipient both histopathologically and microbiologically as quickly and thoroughly as possible is therefore extremely important. If an invasive infection is discovered, the transplant physician should be alerted, because the remaining native lung may harbor the same infection [338]. The presence of an aspergilloma in a potential lung transplant recipient is often considered a contraindication for lung transplantation because of the danger of serious intraoperative bleeding. However, no sound data on this issue exist, especially given the setting of modern surgical techniques. IA of the native lung often requires pneumonectomy [339].

Surveillance cultures for *Aspergillus* are not warranted. The one exception is from lung transplant recipients, in whom the testing of samples should occur. The presence of *Aspergillus* organisms in respiratory cultures mandates inspection for *Aspergillus* tracheobronchitis, anastomotic involvement, and pneumonia. Monitoring for fungal infection after liver transplantation is based on symptoms of infection, and routine monitoring is not necessary.

40.3 Infections Caused by Other Molds

40.3.1 Incidence

In the past few years, the number of infections caused by mold pathogens other than *Aspergillus* has increased. In a multicenter prospective surveillance involving 23 academic centers in the United States, 20% of the mold infections in SOT recipients and up to 56% of IFI-related deaths were caused by molds other than *Aspergillus* species [35, 340]. These mycoses had a higher mortality rate and appeared later than those caused by *Aspergillus*. Thirty-eight percent were diagnosed 6 months after transplantation and 33% at more than 2 years later. Unfortunately, these molds may have variable patterns of susceptibility to current antifungal drugs, which may make their treatment more complex. Some of them are reviewed here.

40.3.2 Mucorales

Mucormycosis or zygomycosis are the names given to the diseases caused by fungi of the order Mucorales. The incidence of mucormycosis complicating SOT seems to be increasing [341]. Although there are no global surveys of the incidence of mucormycosis in SOT patients, its frequency varies widely, from 0.4% to 16% [9, 342, 343], and the onset occurred at a median of 60 days after transplantation [344]. Some authors report a reemergence of zygomycosis and other non-*Aspergillus* mold infections after the introduction of voriconazole and caspofungin in the management of fungal infections [9]. Cases of Zygomicosis have been described after all types of transplantation [9, 344–350]. Almyroudis et al. identified ten cases of zygomycosis in SOT recipients at one center over a 10-year period and reviewed 106 additional cases identified in the literature up to 2002 [344]. In this study, mucormycosis mainly occurred in patients with kidney transplantation ($n=73$), followed by liver ($n=19$), heart ($n=16$), lung ($n=4$), heart and lung ($n=2$) and kidney-pancreas transplantation ($n=2$) [344].

40.3.2.1 Microbiology of Mucorales

Many species have been reported to cause infection in SOT recipients, including *Rhizopus* species, *Mucor* species, *Absidia* species, *Apophysomyces elegans* [351], *Conidiobolus coronatus* [352] and *Cunninghamella bertholletiae* [353, 354]. These organisms typically grow on most media within 2–5 days. However, cycloheximide does inhibit the growth of these fungi, so media containing this compound should not be used when the presence of these fungi is suspected.

The taxonomy of the Zygomycetes was based on the morphologic analysis of the fungus. The differentiation between the genera was accomplished by microscopic examination for the presence and location of rhizoids, the presence of apophyses, and the morphology of the columellae. Other taxonomically relevant features include carbohydrate assimilation and the maximal temperature compatible with growth. The capability to identify Mucorales to the species level is desirable and now is performed by molecular methods [355]. Nowadays molecular methods are crucial for mucormycosis diagnosis because the identification of *Mucorales* species can directly affect epidemiologic studies and treatment strategies, given the differences in antifungal drug susceptibility between species [356, 357]. It may also help the clinician determine whether a fungus found in a subsequent clinical specimen is a different contaminating organism, and it will aid in elucidating the species-specific responses to the new antifungals drugs.

40.3.2.2 Risk Factors

Diabetes mellitus, metabolic acidosis, including that produced in pancreatic transplant recipients with bladder drainage of exocrine secretions; neutropenia; malnutrition; therapy with corticosteroids or deferrioxamine; and acute rejection are predisposing risk factors for this infection [91, 346, 358, 359]. The best risk assessment of zygomycosis in SOT patients has been published in a prospective, multicenter, matched case-controlled study involving consecutive SOT recipients with zygomycosis between September 2003 and July 2008 [9]. The authors describe the clinical characteristics, risk factors, and outcome of SOT recipients with zygomycosis in the era of modern immunosuppressives and newer antifungal agent. In this study, including 50 SOT recipients with invasive zygomycosis and 50 SOT recipients

without zygomycosis, the following variables were significantly associated with a higher risk of infection: renal failure (OR 3.17, $p=0.010$), diabetes mellitus (OR 8.11, $p<0.001$), and voriconazole and/or caspofungin use (OR 4.41, $p=0.033$), whereas the use of the calcineurin-inhibitor, tacrolimus (OR 0.23, $p=0.002$) was associated with a lower risk of zygomycosis [9].

Use of voriconazole, which enhances the virulence of zygomycetes, has been proposed in a number of studies, including hematological patients and SOT recipients, as a risk factor for subsequent zygomycosis [360–363]. Other authors, however, coincided with Singh's finding, in suggesting that is the previous use of any azole or caspofungin, not only voriconazole, what increases the risk of zygomycosis [364].

Remarkably, in the study of Singh et al. [9] tacrolimus was independently associated with a fourfold reduction in the risk of zygomycosis. Although used for its immunosuppressive effects, calcineurin-inhibitors exert certain antifungal role affecting both the virulence and pathogenicity of at least three major opportunistic fungi, *Candida*, *Cryptococcus*, and *Aspergillus* species [365–368]. The precise role of calcineurin inhibitors in the pathogenesis of mucormycosis is not fully elucidated. Both synergistic or additive effect with antifungal agents [368] and an increased inhibition of spore germination, compared with amphotericin B alone [369] have been reported.

40.3.2.3 Clinical Manifestations

Most clinical presentations of these fungi reflect their vascular tropism. These pathogens are notorious for endothelium invasion of blood vessels, which cause infarction in the affected organs and may lead to disseminated infection, which also has extremely high mortality and morbidity rate [370].

In the previous studies, rhinocerebral disease was the most common clinical presentation with one of the following two types: rhinoorbito-cerebral and rhinomaxillary [371, 372]. At present, pulmonary disease is the most prevalent form of zygomycosis in SOT recipients (24–56%), followed by sinus or skin-soft tissue infections (13–31%) and gastrointestinal disease (11–12%) [9, 35, 344, 373, 374]. Disseminated disease occurred in 9–26% and its incidence varied according to the type of transplant recipients: 9–13% in kidney transplantation recipients, 11–20% in heart, 11–25% in lung and 26–55% in liver transplant recipients [9, 344]. A potential explanation for the fivefold higher risk of disseminated disease after liver transplantation are the unique host defense defects or the iron overload in liver transplant recipients [344].

The rhinoorbito-cerebral type begins with fever, swelling, and facial pain [374]. CNS involvement may be precluded by headache, cranial nerve palsies, altered mental status, and seizures. Orbital invasion follows, with the development of cellulitis, ptosis, ophthalmoplegia, the invasion of the ophthalmic and internal carotid arteries, and possibly cavernous sinus thrombosis. Without the early recognition of the syndrome and the prompt initiation of treatment, its mortality is significant. The rhino-maxillary type is less invasive; it involves the sphenopalatine and greater palatine arteries, resulting in thrombosis of the turbinates and necrosis of the palate [359]. This infection can be suspected in a SOT recipient with a clinical presentation of fever, maxillary swelling and edema, and opacification of sinuses on CT scans [350].

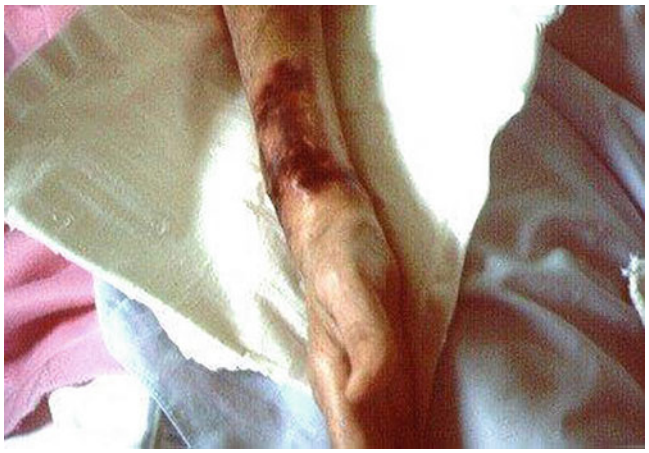

FIGURE 40-2. Cutaneous mucormycosis at intravascular catheter exit site in a heart transplant patient.

Pulmonary disease presents as lung consolidation (22.6%), nodular (25.8%), or cavitary lesions (29%) [373]. Cutaneous and soft-tissue infections present as wound infections, nodular lesions with necrotic ulcerations, venous cannula site infections (Figure 40-2), necrotizing fascitis, and sinus tract infections (e.g., after kidney biopsy) [359, 375–379]. The gastrointestinal form is even rarer and it sometimes is accompanied by gastric or intestinal perforation [359, 380–382]. Widespread dissemination to multiple viscera including the brain may occur [347, 350, 352–354, 383–385].

40.3.2.4 Diagnosis

In patients with solid organ transplantation, early diagnosis of mucormycosis is essential, since delayed or missing diagnosis is related to significant mortality and long-term morbidity. However, diagnosis is often not considered during the initial evaluation of SOT patients as demonstrated in a multicenter study performed in France in which the median time from first symptoms to mucormycosis diagnosis was 6 weeks [386].

The hallmarks of disease caused by the Mucorales are vascular invasion and tissue necrosis; black eschars and discharges should arouse the suspicion of this disease. These manifestations occur only after the patient have been infected for some time, so attempts to make a diagnosis of mucormycosis should not await the development of necrotic areas. The diagnosis depends on demonstration of the organ-

ism in the tissue of a biopsy specimen. The microscopic examination of scotch tape touch preparations over the "hairy pus" that is sometimes present in skin areas of mucormycosis may provide an immediate diagnosis.

If mucormycosis is suspected, then a CT scan of the brain, sinus, and chest should be performed, eventually associated with a bronchoscopy if the CT scan of the chest shows abnormalities [387].

An histopathologic examination of the biopsies typically show broad (10–20 mm in diameter), nonseptate hyphae with branches that occur at right angles. Rarely, septae can be visualized. The appearance of Mucorales hyphae in tissue is different from that of *Aspergillus*, *Fusarium,* and *Pseudallescheria* species, as this latter organisms appear as thinner, more regularly shaped fungal elements with more frequent, acute-angle branching. These hyphae are also septate.

The identification of the genus and species requires culture of tissue and assessment of the morphology of the fungal growth. However, in a significant number of cases, cultures results are negative. For reasons that are not altogether clear, the agents of mucormycosis can be difficult to isolate from infected tissue, and they rarely appear in blood culture. Grinding the tissue in the microbiology laboratory is not recommended because the hyphae may be destroyed, possibly preventing the isolation of the fungus from the sample. As mentioned, at present molecular methods are used for species identification [357].

Finally, although not enough clinical data are available, the negativity of β-glucan in a SOT patient with a suspicion of invasive fungal infection can support the diagnosis of mucormycosis [387]. However, because of the high prevalence of mixed fungal infection, clinicians should keep in mind that a positivity of β-glucan or galactomannan do not exclude the diagnosis of mucormicosis [388].

40.3.2.5 Treatment

Neither the classical azoles, ketoconazole, itraconazole, and fluconazole, nor voriconazole have a potential role in the treatment of mucormycosis. The standard therapy for invasive mucormycosis is amphotericin B [389]. Outcome appears to be better with lipid formulations of amphotericin B than with amphotericin B deoxycholate. Singh et al. [9] reported a fourfold higher success rate in patients receiving liposomal amphotericin B, even when controlled for other variables influencing outcomes such as renal failure, disseminated disease, and surgical resection. For primary treatment, liposomal amphotericin B with a usual dose of 5 mg/kg/day is recommended for most patients. However, some experts recommend the administration of higher than usual doses (i.e., as high as 10/mg/kg/day), if needed, in order to control the infection [350].

Posaconazole should be considered as maintenance therapy in persistently immunocompromised patients or as salvage therapy in patients who have disease refractory to or who are intolerant of standard antifungal therapy [387]. Its efficacy as salvage therapy for zygomycosis has been reported in different hosts, including patients with SOT [390–392] and in Singh series 60% of the patients receiving posaconazole as primary therapy had a successful outcome [9]. Of note, posaconazole has important drug–drug interactions with immunosuppressive agents commonly used for SOT and serum levels concentrations should be monitored in order to optimize its variable pharmacokinetic [345].

Although echinocandins have typically demonstrated little activity against zygomycetes and breakthrough infections have been reported in patients receiving these agents, there is a considerable interest in the use of echinocandins as part of combination antifungal therapy for zygomycetes [393, 394]. We evaluated the in vitro effect of combining posaconazole and caspofungin against 12 clinical zygomycetes showing that, although the caspofungin MICs were all above serum drug levels, the combination proved to be synergistic in all strains [395].

The success of treatment is dependent on an early diagnosis, the suppression of predisposing factors, the extension of the disease and the possibility of surgical treatment [9, 345, 350, 373, 396]. Appropriate surgical treatment was reported to be an independent predictor of successful outcome in SOT patients with rhino-orbital-cerebral [374] and pulmonary zygomycosis [373]. It consists of the early extensive debridement of necrotic tissue, ideally until the surgical margins are free of the infection [350]. A pulmonary wedge resection may be sufficient, however, at times, a lobectomy is required to eradicate a confined lung mucor infection, which is associated with a mortality rate of 65% [350, 396]. A surgical approach is recommended even if complete resection is not feasible.

40.3.3 Dematiaceous Fungi

40.3.3.1 Microbiology

Dematiaceous is the term that is applied to septate molds with dark walls in culture, an appearance that the walls do not necessarily have in tissue. More than 100 species that belong to at least 57 genera are known to be agents of infection. *Alternaria*, *Bipolaris*, *Curvularia,* and *Exserohilum* are the most frequently isolated genera, while others (*Chaetomium*, *Cladophialophora*, *Cladosporium*) have been reported only anecdotally. These saprophytic fungi are widely distributed in the environment. One large prospective study on dematiaceous infections in SOT recipients analyzing clinical characteristics, site of infection, and outcome has been recently published [397].

40.3.3.2 Incidence

Data from the USA was offered in the multicenter register TRANSNET, in which 2.5% of invasive fungal infections among SOT recipients were caused by phaeohyphomycosis

[397]. One American institution reported an overall incidence of infection due to dematiaceous fungi of 0.7% with a higher rate of infection among kidney/pancreas transplant patients (3.6%) [398]. In these studies, the time from transplantation to onset of infection varied widely, ranging from 19 days to 11 years [397, 398].

40.3.3.3 Clinical Manifestations

The infections caused by these fungi include chromoblastomycosis, phaeohyphomycosis, and mycetoma, although the latter could be caused by other molds or by *Actinomyces* species. Mycetoma and chromoblastomycosis are infections that are localized in the skin and subcutaneous tissue; phaeohyphomycosis comprise a heterogeneous group of infections that range from superficial, cutaneous, or subcutaneous infections to disseminated invasive disease. Some of the infections caused by these fungi are summarized in Table 40-4.

Clinically, the most common manifestations are cutaneous and subcutaneous disease, followed by pulmonary and invasive sinus infection [397]. Disseminated disease, involving more than one noncontiguous site of infection, is present in more than half of the patients, whereas bloodstream or central nervous system infection are uncommon among SOT patients [397]. The survival rate among patients with soft-tissue infections is more than 90% [397], whereas that for patients with invasive infections is lower, at approximately 50% [50, 399]. The only patient with CNS infection died [397].

40.3.3.4 Diagnosis

Melanin in the hyphal wall can be stained with the Masson-Fontana dye, allowing differentiation from other nonmelanin containing fungi. With hematoxylin and eosin staining of tissue, the fungus has a golden brown hyphal wall. The hyphae are irregular in diameter and are septate. Chains of yeast-like cells may be seen with the presence of some species. The irregular diameter and bulbous swellings of these hyphae may help distinguish them from *Aspergillus,* but culture is essential for diagnosis. Molecular methods are very helpful for the correct identification at species level [400].

40.3.3.5 Treatment

Surgical excision or debridement is recommended whenever possible, and represents a mainstay of therapy [401]. For both soft-tissue lesions and single brain abscesses, the necessity of achieving disease-free resection margins to prevent the recurrence of infection should be emphasized [402].

The recognition of infection due to dematiaceous fungi is important because these infections, unlike IA, may be more accessible to therapy. These fungi are generally susceptible to itraconazole, which is the preferred treatment option, given the wide clinical experience with this drug. However, because of varied resistance pattern among species [398, 403], antifungal susceptibility data should be obtained for all specimens, if feasible. Recently, the European Society of Clinical Microbiology and Infectious Diseases published guidelines for treatment of infection caused by dematiaceous fungi and recommended as first line options voriconazole, posaconazole, itraconazole, and amphotericin B [105, 401]. Voriconazole and amphotericin B should be preferred for the treatment of central nervous system infections because of their consistent ability to achieve good cerebrospinal fluid concentrations [105, 397].

After a recurrence, long-term itraconazole therapy may be used following repeated surgical drainage to help prevent a new recurrence. Combination therapy such as amphotericin B with 5-FC (when the fungus is susceptible to the drug) [404] has been advocated for CNS lesions, particularly for lesions that are poorly resectable or are unresectable. Posaconazole has been employed successfully as salvage therapy in hematological patients with refractory invasive mold infections, including *Alternaria* spp. [405].

In vitro susceptibility to echinocandins has been described for the *Curvularia*, *Exophiala,* and *Fonseca* species, although their role for the treatment of these infections remains to be clarified.

40.3.4 Hyalohyphomycosis

40.3.4.1 Microbiology

The term hyalohyphomycosis has been proposed for the opportunistic mycotic infections that are caused by fungi whose tissue form consists of hyaline hyphal elements. Fusariosis accounts for approximately ~1% of fungal infections in SOT recipients, being lung transplant recipients the most commonly affected [340].

Infections in SOT recipients caused by various fungi of this type in SOT recipients are summarized in Table 40-5.

40.3.4.2 Clinical Manifestations

Fusarium Species

Unlike fusarial infection in patients with hematologic malignancies or bone marrow transplantation, fusarial infection in SOT recipients tends to be localized [406, 407], it occurs later in the posttransplantation period, and it has a better outcome [408]. The skin lesions usually appear as cellulitis, ulcers, papules, or as deeply set, painful nodules. They may initially be flat (macular) with a central pallor, but they later become raised, erythematous, and necrotic. Reports on fusariosis in kidney ($n=6$), lung ($n=2$), combined heart–liver

TABLE 40-4. Infections by dematiaceous fungi in solid organ transplant recipients

Type of fungi	Type of transplant	Clinical presentation	Reference
Alternaria alternata	Heart	Cutaneous	[472]
	Kidney	Cutaneous	[473]
	Kidney	Cutaneous	[474]
	Liver	Cutaneous	[475]
Alternaria infectoria	Heart	Cutaneous	[476]
	Kidney	Disseminated	[477]
	Kidney	Cutaneous	[478]
	Liver	Cutaneous	[479]
Alternaria species	Kidney	Cutaneous	[478, 480, 481]
	Liver	Cutaneous	[482]
Aureobasidium pullulans	Liver	Lung	[483]
Bipolaris hawaiiensis	Kidney	Nasal	[484]
Chaetomium globosum	Kidney	CNS	[485]
Cladophialophora bantiana	Heart	Disseminated	[486]
	Liver	CNS	[487]
	Lung	CNS	
	Lung	CNS	[488]
	Kidney	CNS	[489]
	Kidney	Cutaneous	[490]
Cladophialophora carrionii	Kidney-Pancreas	Cutaneous	[491]
Cladosporium trichoides	Liver	CNS	[492]
Dactylaria constricta	Heart	Lung	[493]
	Kidney	Disseminated	[494]
Dactylaria gallopava	Liver	CNS	[495, 496]
	Lung	Soft tissue	[497]
Exophiala jeanselmei	Heart	Cutaneous	[498, 499]
	Kidney	Cutaneous	[500–508]
	Lung	Cutaneous	[509]
	Lung	Disseminated	[510]
Exophiala pisciphila	Liver	Cutaneous	[117]
Exophiala species	Heart	Cutaneous	[511]
	Kidney	Cutaneous	[512]
Ochroconis gallopavum	Heart	Lung	[513]
	Renal	Disseminated	[514]
	Lung	Cutaneous	[515]
Phialemonium obovatum	Kidney	Peritonitis	[516]
Phialemonium species	Kidney	Cutaneous	[516]
Phialophora bubakii	Kidney	Cutaneous	[517]
Phialophora gougeroti	Kidney	Cutaneous	[518]
Phialophora parasitica	Kidney	Cutaneous	[402, 519]
Pleurophoma species	Heart	Cutaneous	[520]
Pleurophomopsis lignicola	Kidney	Cutaneous	[521]
Scopulariopsis brumptii	Liver	CNS	[446]
Scytalidium dimidiatum	Kidney	Cutaneous	[522]
Veronaea bothryosa	Liver	Cutaneous	[523]

Abbreviations: *CNS* central nervous system.

($n=1$) and liver ($n=3$) transplantation recipients have been published. Presentation forms include seven cases of localized skin infections, three disseminated infections (one endocarditis and one with liver abscesses), one peritonitis, and 1 lung abscess [406–417].

Paecilomyces Species

Paecilomyces infection has been described in ten SOT recipients [244, 418–426]. The skin and soft tissues were affected in nine cases, and, in one pediatric lung transplant recipient, the fungus was isolated from the respiratory tract.

TABLE 40-5. Infections caused by hyaline fungi in solid organ transplant recipients

Type of fungi	Type of transplant	Clinical presentation	Reference
Acremonium species	Heart	Bone	[524]
	Heart	N.A.	[525]
	Heart	Mycetoma	[526]
	Kidney	Cutaneous	[527]
	Kidney	Lung	[528]
	Kidney	Mycetoma	[529]
Fusarium species	Heart–liver	Cutaneous	[408]
	Kidney	Cutaneous	[407]
	Kidney	Cutaneous	[406]
	Kidney	Fungemia	[411]
	Kidney	Peritonitis	[412]
	Kidney	Cutaneous	[415]
	Kidney	Cutaneous	[417]
	Liver	Liver abscesses	[413]
	Liver	Cutaneous	[414]
	Liver	Cutaneous	[416]
	Lung	Endocarditis	[410]
	Lung	Lung cavitation	[409]
Paecilomyces species	Heart	Cutaneous	[420, 426]
	Kidney	Cutaneous	[425]
	Lung	Airway colonization	[422]
Penicillium species	Kidney	Cutaneous	[462]
	Kidney	Intestinal	[530]
	Kidney	Disseminated	[531]
	Lung	Lung infection with mediastinal lynphoadenopathy	[515]
PhaeoAcremonium parasiticum	Kidney	Cutaneous	[532]
	Liver	Endocarditis	[533]
	Lung	Lung cavitation	[534]
Scedosporium species	Kidney (20)	Mediastinitis (3%)	[45]
	Lung (14)	Pulmonary involvement (46%)	[433]
	Heart (17)	Skin involvement (26%)	
	Liver (11)	CNS involvement (25%)	
	Small-bowel (4)	Fungemia/Disseminated infection (50%)	
	Multivisceral		
Scopulariopsis species	Heart	Cutaneous	[445]
	Liver	CNS	[446]
	Liver	Cutaneous	[443, 444]
Trichoderma species	Kidney	Disseminated	[535]
	Liver	Sinusitis	[536]
Trichoderma viride	Liver	Surgical site infection	[537]

Abbreviations: *CNS* central nervous system, *N.A.* not addressed.

Scedosporium Species

Human infection with *Pseudallescheria boydii* (the anamorph state of *S. apiospermum)* or with *S. prolificans* can produce the following two distinct diseases: mycetoma and pseudoallescheriasis. Mycetoma is a chronic subcutaneous infection that is characterized by the production of grains. Pseudallescheriasis includes all other infections caused by *Scedosporium* species [427–432].

Scedosporium species now account for ~25% of all non-*Aspergillus* mold infections in SOT recipients [8]. In a recent article, Husain et al. [45] published a series of 80 transplant recipients with *Scedosporium* infections (57 SOT and 23 HSCT recipients). Among SOT recipients, 83% of the infections were due to *S. apiospermum* and 19% to *S. prolificans.* Dissemination occurred in 46% of the patients, CNS involvement in 29%, lung infection in 43% and cutaneous disease in 31%. Fungemia was significantly more common with *S. prolificans* than with *S. apiospermum* infections (57% vs 8%, $p<0.001$). The death rate ranged from 54% to 73%, a figure that was nearly 100% in patients with disseminated disease, CNS involvement, fungemia, and renal failure [45].

More recently, Johnson et al. [433] reported a series of 11 SOT patients infected by *S. apiospermium* (55%) or *S. prolificans* (45%). Overall, 55% (6/11) scedosporiasis occurred

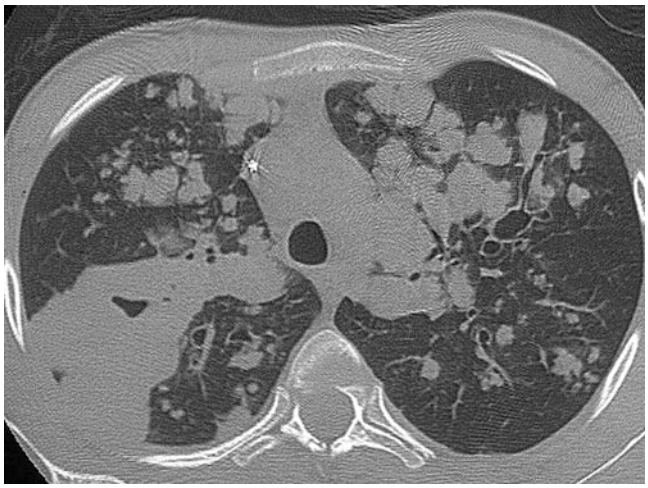

FIGURE 40-3. Invasive lung infection caused by *Scedosporium prolificans* in a young liver transplant recipient with cystic fibrosis. The patient died despite therapy with liposomal amphotericin B and voriconazole.

in lung transplant patients, whereas 18% (2/11) were diagnosed in multivisceral transplants. Interestingly, 36% of the patients were previously colonized by the same species and developed disease despite receiving preemptive voriconazole therapy either alone or combined with other antifungal agents. Scedosporiasis occurred earlier among patients who were colonized in the pretransplantation period compared with those who were not (15 vs 217 days). Clinical manifestations include pneumonia, mediastinitis, and fungemia/disseminated disease. Patients who died (6/11, 55%) had an earlier diagnosis of scedosporiasis after transplantation and were more likely to have clinical manifestations other than pneumonia.

In recent years, *Scedosporium* infections have been observed to occur later after transplantation (4–6 months) than before [45, 434]. It is argued that the more frequent use of antifungal prophylaxis after transplantation could have delayed the onset of these infections and exerts a selective pressure favoring the emergence of these resistant pathogens [433, 435–437]. The radiological and histopathologic appearance of scedosporidiosis is indistinguishable from infections caused by other molds (Figure 40-3), with the exception of a higher tendency to involve the CNS. Definite diagnosis depends therefore on fungal culture. New molecular diagnostic methods have been also employed to distinguish *S. apiospermum* from *S. prolificans* [438, 439].

The early diagnosis of this infection is essential because S. *apiospermum* is resistant to amphotericin B, and *S. prolificans* is also resistant to most currently available antifungal agents [431, 432, 440]. Based on previous experiences [45, 433, 441], current treatment of scedosporiasis comprise voriconazole and prompt surgical debridement, if the latter is feasible [45]. A synergistic effect between voriconazole and terbinafine has been shown in vitro and this combination should be considered in case of a disease due to *S. prolificans.*

Scedosporium can colonize the sinuses and airways of patients with underlying pulmonary diseases and is the second most frequent filamentous fungus, after *A. fumigatus*, isolated from the sputum in cystic fibrosis patients with a prevalence of >8% [442]. Pretransplant colonization should be considered as an indication for double-lung transplantation in candidates for lung transplantation. In all SOT recipients, colonization by *Scedosporidium* should not be ignored and prophylaxis or suppressive therapy with voriconazole should be considered in these patients [442].

Scopulariopsis Species

Scopulariopsis infection is also extremely rare in the SOT population. Eight cases have been described [443–450], three in liver transplant recipients, three in lung transplant recipients, one in a heart and one in a lung-heart transplant recipient. Three patients presented with skin or subcutaneous tissue involvement, which was cured. Of two patients presenting with respiratory tract infections, one survived receiving combination antifungal therapy with posaconazole and terbinafine, whereas the other died. One patient, who presented with a CNS abscess, died despite treatment with amphotericin B and miconazole, and in the other fatal case *Scopulariopsis Acremonium* was identified in the pericardial biopsy after the patient died. Finally, a heart–lung transplant patient developed disseminated disease due to *Microascus cirrosus* and died 4 weeks after transplantation despite antifungal combination therapy with voriconazole and caspofungin.

40.3.4.3 Diagnosis

The diagnosis is most commonly made by culturing the organism from infected sites. In contrast to aspergillosis, in which the blood cultures are nearly always negative, fungemia due to *Fusarium* or *Scedosporium* species is not rare. These fungi are indistinguishable from *Aspergillus* in histopathologic studies (i.e., they all present with dichotomously branching septate hyaline hyphae). Therefore, it is necessary to isolate them from a culture for their identification to proceed. The identification is based chiefly on the morphologic characteristics of the asexual structures produced by the molds in culture.

40.3.4.4 Treatment

Treatment of infections caused by mold pathogens other than *Aspergillus* can be very challenging. Because randomized controlled trials have not been published yet, data regarding the optimal management of such infections are limited. As with any opportunistic infection, the first step in the therapeutic strategy should be to correct the underlying risk factors. The doses of immunosuppressive drugs, including steroids, should be decreased, if possible. The recovery of the immune status of the patient is vital since treatment with conventional antifungal drugs (e.g., amphotericin B, the

azoles) are less efficacious, either because these species are more resistant to drugs or because they have a more aggressive clinical evolution.

The optimal treatment regimen for fusarial infections is unknown. This fungus is uniformly resistant to 5-fluorocytosine, and it is usually resistant to itraconazole as well. Surgical resection, when this is possible, and prolonged treatment with a lipid formulation of amphotericin B at a dose of 5 mg/kg has been the standard of care [408]. The clinician must remember that susceptibility to amphotericin B varies. Therefore, some strains have been refractory to this treatment; in these cases the newer compound should be tried. At present, voriconazole is, in many experts' opinions, the drug of choice for *Fusarium* infections, although more data in SOT are required and susceptibility studies should be performed in each case. Salvage therapy with posaconazole was found to be effective and safe in two SOT patients with invasive fusariosis intolerant of or failing other antifungal therapies [392].

The susceptibility of *Paecilomyces* species to the currently available antifungal agents is highly variable among the different species. Therefore, precise fungal identification and in vitro susceptibility testing are mandatory. When immediate antifungal therapy is required, amphotericin B and 5-fluorocytosine should be considered for infections caused by *Paecilomyces variotti*, whereas the imidazoles might have more efficacy in treating *P. lilacinus* and *Paecilomyces marquandii* [424]. In one heart transplant recipient with leg nodules caused by *P. lilacinus*, the infection was successfully treated with terbinafine [420]. Another heart transplant patients who presented with mixed cutaneous infection due to *M. chelonae* and *Paecilomyces* species was successfully treated with sequential itraconazole and terbinafine [426]. Treatment with traditional antifungal drugs often fails. Voriconazole has demonstrated good activity in both cutaneous and ocular infections in the few cases in which this drug has been used. The new triazoles ravuconazole and posaconazole show good in-vitro activity against *P. lilacinus* and could be promising therapeutic alternatives [451].

Response to therapy in patients with scedosporiasis is strictly dependent on the site of infection, extent of dissemination, and immune status of the host. Generally, outcome is more favorable in patients who have localized infection and receive a prompt surgical debridement [401]. Antifungal therapy of infections by *P. boydii* (the anamortph of *S. apiospermum*) has not been established. Reports indicate that it has in vitro and clinical resistance to amphotericin B [245], 5-flucytosine and terbinafine. Success with itraconazole and voriconazole has been reported [45, 452]. Surgical debridement is an important adjunct in the treatment of pseudallescheriasis of the soft tissue, bone, joint and pleural, and paranasal sinuses, although it is not curative in and of itself. Voriconazole seems to be a good therapeutic option for improving surgical results, an in vitro susceptibility to caspofungin has also been described [453].

Infections caused by *Scedosporium prolificans* are quite difficult to treat because this fungus appears to be intrinsically resistant to most of the currently available antifungal agents [436]. These infections may respond to azole antifungal agents [454] or to the combination of terbinafine with the azoles voriconazole, miconazole, and itraconazole [455].

40.3.5 Other Fungi: Dermatomycoses

40.3.5.1 Microbiology

Superficial fungal infections (dermatomycoses) include some common conditions such as ringworm or dermatophytosis; pityriasis versicolor, which is caused by yeasts, such as *Malassezia* species; and some rare disorders, such us tinea nigra, a superficial form of phaeohyphomycosis caused by *Hortaea (Exophiala) werneckii*.

The dermatophytes are molds that can invade the stratum corneum of the skin or other keratinized tissues derived from epidermis, such as the hair and nails. They may cause infection at most skin sites, although the feet, groin, scalp, and nails are most commonly affected. Nail fungal invasion is termed onychomycosis. The three genera of pathogenic dermatophyte fungi are as follows: *Trichophyton*, *Microsporum,* and *Epidermophyton*.

Infections caused by *Microsporum* and *Trichophyton* have been described in the following SOT recipients: heart [456, 457]; heart–lung [458], liver [459] and kidney [460–467]. Pityriasis versicolor is the most frequent dermatomycoses in SOT population, and it has a higher prevalence than that encountered in the normal population (Figure 40-4). The prevalence of onychomycosis is similar to that observed in the immunocompetent population. The probability of dis-

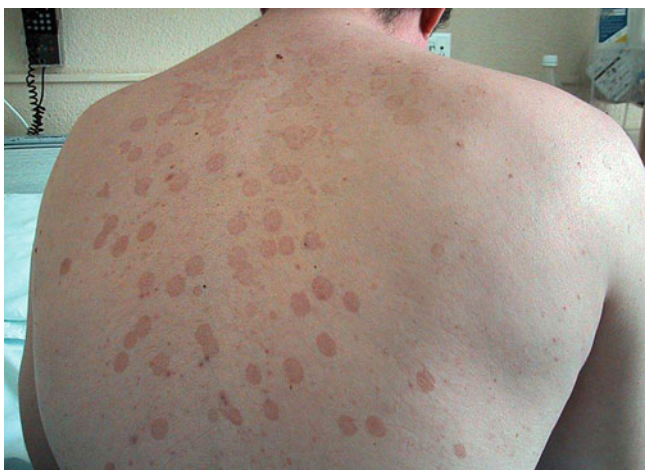

FIGURE 40-4. Pytiriasis versicolor in a heart transplant patient.

ease appears to increase with the length of time after transplantation, as does the incidence of mixed or simultaneous fungal infections in the same patient [462].

40.3.5.2 Risk Factors

As in all other mold infections, the chronic immunosuppressive state of SOT recipients makes their skin more liable to fungal infections [457, 462].

40.3.5.3 Clinical Manifestations

The typical tinea lesion is an annular scaling patch with a raised margin that has a variable degree of inflammation, in which the center is usually less inflamed than the edge. The clinical appearance of the infection sometimes varies with the site, the fungal species involved, and the host's immune response. At times, the lesions are inflammatory, and. in some cases, large pustular lesions may develop. Lesions may become chronic.

The clinical appearance is altered in immunocompromised individuals. In these patients, the fungi may invade subcutaneous tissues via the lymphatics, usually causing clusters of granulomas, lymphedema, and draining sinuses. Deep dermatophyte infections may extend further, involving draining lymph nodes or other sites, including the liver and brain; these may be fatal. In SOT recipients, specifically extensive dermal disease (Majocchi's granuloma) [457, 468–470] and primary invasive cutaneous disease have been reported [459, 464].

As in nonimmunosuppressed hosts, the most common clinical pattern of onychomycosis in SOT recipients is distal and subungual onychomycosis, in which the nail plate is invaded from the distal and lateral borders. Associated thickening of the nail, which becomes white, yellow, or brown, is usually observed.

40.3.5.4 Diagnosis

The laboratory diagnosis of dermatophytosis depends on the examination and culture of scrapings or clippings from lesions. Sampling the edge of skin lesions and infected nails is important. The material should be allowed to soften in 10–20% potassium hydroxide before it is examined under the microscope. Fungal hyphae can be seen as chains of arthrospores in cleared scales or clippings.

Scrapings or nail clippings may also be cultured. The primary isolation is carried out at room temperature, usually on Sabouraud's agar containing antibiotics and cycloheximide, an antifungal agent that suppresses the growth of environmental contaminant fungi. In the case of nail disease, media without cycloheximide should be used, because certain fungi, such as *Scytalidium*, that may infect nails are sensitive to the latter. Most dermatophytes can be identified within 2 weeks. The identification mostly depends on the gross colonial and microscopic morphology.

40.3.5.5 Treatment

Itraconazole, ketoconazole, terbinafine, and griseofulvin are used as oral therapy for cutaneous and subcutaneous dermatomycoses. CsA levels should be monitored during ketoconazole therapy because they usually increase and may cause nephrotoxicity.

The oral agent terbinafine, an allylamine structurally related to naftifine, is effective in the treatment of ringworm, including onychomycosis; its efficacy is comparable to that of the azoles derivatives [457]. It is 99% protein bound. Terbinafine is metabolized in the liver and it has an initial half-life of 12 h. The drug accumulates in skin, nails, and fat. Probably because of its accumulation in fat and subsequent release, terbinafine persists in plasma for 4–8 weeks after dosing, and it has a terminal half-life of 200 to 400 h. This clearance is slowed in the presence of liver or renal impairment. Rifampin markedly increases terbinafine clearance, while cimetidine modestly decreases it. Terbinafine induces CsA metabolic degradation, which, however, is of little clinical significance. The CsA levels, however, should be controlled during treatment with this drug [471]. Side effects, principally gastrointestinal and taste complaints, rarely limit therapy. Hepatitis is an uncommon side effect. Other approaches, such as topical agents and excisional procedures, may also be used as complement of systemic antifungal drugs.

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41
Endemic Mycoses After Hematopoietic Stem Cell or Solid Organ Transplantation

Carol A. Kauffman and Marisa H. Miceli

41.1 Introduction

The three major endemic mycoses that cause infections in transplant recipients are histoplasmosis, coccidioidomycosis, and blastomycosis. When compared with opportunistic fungi, the endemic mycoses are responsible for many fewer infections among transplant recipients [1–3]. The endemic mycoses exhibit temperature and tissue dimorphism. In the environment and at temperatures below 35 °C in the laboratory, these organisms are molds; at body temperature, they exist as yeasts (*Histoplasma capsulatum* and *Blastomyces dermatitidis*) or spherules (*Coccidioides immitis* and *Coccidioides posadasii*).

H. capsulatum, Coccidioides species, and *B. dermatitidis* occur naturally in the environment only in specific geographic areas. Thus, an infection will occur only if the patient has been in that specific area at some time in his or her life. Many patients who are markedly immunosuppressed do not feel well enough to participate in outdoor activities, and thus their exposure to the endemic mycoses is limited. However, the conidia of *Coccidioides* species are easily aerosolized, and coccidioidomycosis can be acquired simply by living in or merely passing through the endemic area [4, 5].

The exposure to one of the endemic mycoses may have occurred years, even decades earlier. These organisms have the ability to remain dormant in the body after the initial infection. Years later, when immunosuppression occurs for a variety of different reasons, the organisms reactivate and proliferate, causing disease. The prototype organism that has been documented most clearly as a cause of reactivation infection is *H. capsulatum* [6]. *Coccidioides* reactivation has been postulated to cause infection early in the post-transplantation period [7], and reactivation of *B. dermatitidis* also has been thought to account for some infections in the post-transplant period [8]. Because these infections are unusual outside the endemic area, the diagnosis can be easily missed and appropriate treatment not given in patients currently living in an area in which these organisms are not endemic. A careful history of travel and residence should be confirmed before any transplantation procedure so that the clinician is aware of all possible post-transplantation infectious complications.

Both *H. capsulatum* and *Coccidioides* species, but not *B. dermatitidis,* have been noted to have been transmitted with the donor organ, reflecting their ability to remain latent in many different tissues and to reactivate with immunosuppression [3, 9–17]. In several of these cases, the donor had spent time in the endemic area but the recipient had never even traveled to those areas.

These fungi are true pathogens in that they cause disease in healthy people, as well as immunosuppressed hosts. The severity of infection depends on the extent of the exposure (the number of conidia inhaled) and on the immune state of the host. Following inhalation, hematogenous dissemination occurs frequently, even in the normal host, but it is almost always a silent event, with no evident disease. However, immunosuppressed patients are more likely to develop severe symptomatic disseminated infection at the time of initial infection [18–20].

The agents of choice for treatment of the endemic mycoses are a lipid formulation of amphotericin B for those with severe infection and itraconazole or another azole after the patient has responded to amphotericin B. Primary therapy with an azole is used occasionally in a transplant recipient who has only localized infection, and azoles are used for prophylaxis to prevent primary infection and suppressive therapy to prevent relapse.

41.2 Histoplasmosis

41.2.1 Epidemiology

The endemic area for *H. capsulatum* encompasses the Mississippi and Ohio River valleys and focal areas along the eastern coast of the USA, many areas throughout Central and South America, and focal areas in Africa and Southeastern Asia. The organism grows best in soil that contains high concentrations of nitrogen, which is found commonly under roosts for birds and bats. Point-source outbreaks most often

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involve healthy individuals who have participated in a variety of activities, including cleaning attics and barns, demolishing old buildings, landscaping, and spelunking, but can also occur in an urban setting, as happened in Indianapolis several decades ago, in which more than 120,000 residents were infected [21, 22].

Histoplasmosis is the most common endemic mycosis reported in solid organ transplant recipients [1, 3, 10, 16, 17, 23–32]. The 12-month cumulative incidence rate for histoplasmosis among solid organ transplant recipients was reported to be 0.1% in the 5-year, multi-center, transplant-associated surveillance network (TransNet) study [3]. Fewer cases of histoplasmosis have been reported to occur after hematopoietic stem cell transplantation [2, 3, 20, 33–35]. It is likely that histoplasmosis is the most frequently reported endemic mycosis in transplant recipients because of the wide geographic distribution of this organism and the high attack rate among inhabitants residing in the endemic area.

41.2.2 Pathogenesis

Infection follows aerosolization of the microconidia of *H. capsulatum* into the lungs where they are phagocytized by alveolar macrophages and then convert to the yeast phase inside the cell. The organism routinely disseminates hematogenously to the organs of the reticuloendothelial system before cell-mediated immunity arms the host's macrophages to kill the intracellular yeasts [36]. The primary host defense against *H. capsulatum* is cell-mediated immunity with little contribution from the humoral or neutrophil defense mechanisms; thus, patients with cell-mediated immunodeficiencies, such as transplant recipients, are at the highest risk of severe infection.

The occurrence of histoplasmosis is bimodal, with some cases occurring within the first 6 months (40% of cases in the TransNet study) and others occurring as long as 18–20 years after transplantation [3, 16]. As most solid organ transplant recipients continue on some type of immunosuppression, the period at risk for an endemic mycosis persists long after the immediate post-transplant period.

H. capsulatum has the propensity to persist in a latent state for years and then to reactivate when immunosuppression intervenes. Reactivation infection has been noted in some transplant recipients, but differentiating reactivation from new infection in patients who live in the endemic area is difficult. Infection could as easily be due to a new infection as to reactivation.

Transmission of *H. capsulatum* from the donor organ has been documented in a small number of patients and suspected in others because of early onset of disease after transplantation [3, 10, 16, 17, 37–39]. In a few patients, *H. capsulatum* was found in the donor organ, and the recipients were pre-emptively treated with antifungal agents [3, 17].

41.2.3 Clinical Manifestations

Transplant recipients can have primarily pulmonary manifestations [3, 17, 23, 24, 29, 30] or more commonly, disseminated infection [3, 10, 17, 24, 27, 28, 30–34, 37–39]. Pulmonary infection presents with fever, chills, cough, and shortness of breath and frequently progresses to marked dyspnea and hypoxemia. Chest radiographs most often show diffuse bilateral infiltrates (Figure 41-1). Mediastinal or hilar lymphadenopathy, common seen in acute pulmonary histoplasmosis in healthy hosts, is rarely seen in immunosuppressed patients.

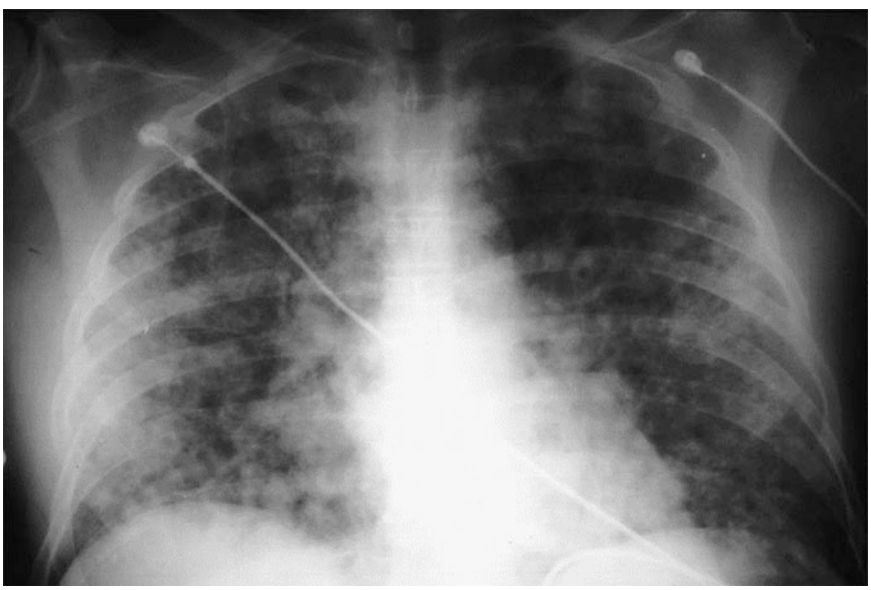

FIGURE 41-1. Bilateral pulmonary infiltrates in a patient who had received a kidney transplant 2 years before. *H. capsulatum* grew in bronchoalveolar lavage fluid and in blood cultures.

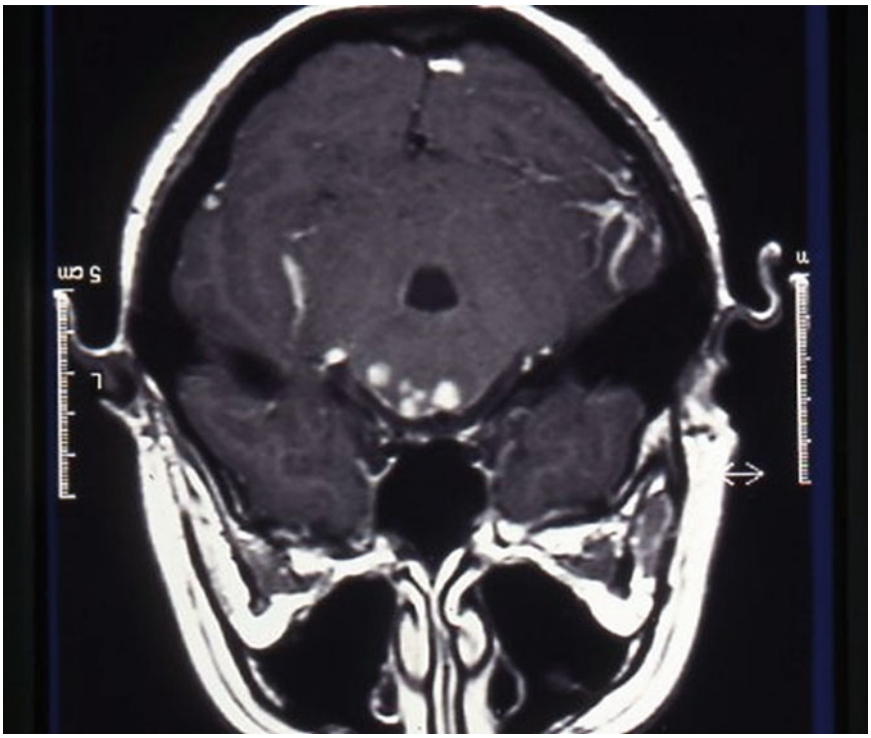

FIGURE 41-2. Enhancing brain lesions in a patient who had meningoencephalitis due to *H. capsulatum.*

The manifestations of disseminated histoplasmosis are protean, but most patients present with fever, chills, fatigue, anorexia, and weight loss. Hepatosplenomegaly, mouth ulcers, and skin lesions should be sought. The mucous membrane lesions are usually painful and nonhealing ulcers; the cutaneous manifestations include papules, pustules, plaques, ulcers, abscesses, and cellulitis. Abnormal liver enzyme values, especially elevated alkaline phosphatase, and pancytopenia are common. Gastrointestinal involvement is manifested by diarrhea and abdominal pain; ulcerations have been noted diffusely throughout the small bowel and colon. Severe disseminated histoplasmosis can be associated with DIC and life-threatening hemophagocytic syndrome [31]. Central nervous system involvement is not common, but probably occurs more often in immunosuppressed patients; it usually is characterized by lethargy, headache, and confusion. Intracranial mass lesions that are best visualized on magnetic resonance imaging can occur with or without meningitis (Figure 41-2).

The mortality of histoplasmosis in transplant recipients is reported to be 9–15% in recent series [3, 16, 24]. In cases in which the diagnosis is delayed, the mortality is high [3, 33, 34, 38]. Not surprisingly, this is especially true of patients who present outside the endemic area for histoplasmosis. When the diagnosis is made early and appropriate therapy is initiated, the mortality is less than 10%. *H. capsulatum* is very susceptible to amphotericin B, and most patients respond quickly to appropriate therapy.

41.2.4 Diagnosis

The definitive diagnosis of histoplasmosis is made by growing *H. capsulatum* from sputum, blood, other body fluids, or biopsy tissue [6]. *H. capsulatum* grows very slowly, a decided drawback in an immunosuppressed patient. The yield from blood cultures is enhanced by using the lysis-centrifugation system (Isolator tube system, Wampole Laboratories); automated blood culture systems, such as BacT/Alert and Bactec, have a lower yield and take a longer time to show growth of *H. capsulatum* [40]. Although growth in culture provides proof of the cause of the illness and is extremely important, it often merely confirms the correct diagnosis in a patient for whom treatment has already been initiated.

Serological assays (complement fixation and immunodiffusion) are useful tests for the diagnosis of histoplasmosis in normal hosts. The development of complement fixation antibody or the appearance of specific bands on the immunodiffusion assay, especially when baseline results are negative, can be quite helpful. However, these tests are often not positive in immunosuppressed patients who cannot mount an effective antibody response [41].

The detection of *H. capsulatum* capsular polysaccharide by enzyme immunoassay (EIA) on urine and serum is an extremely useful test in patients who have disseminated histoplasmosis [42, 43]. This assay has assumed an increasingly important role in the diagnosis of histoplasmosis in transplant recipients. False positive assays occur primarily with blastomycosis and less commonly with coccidioidomycosis.

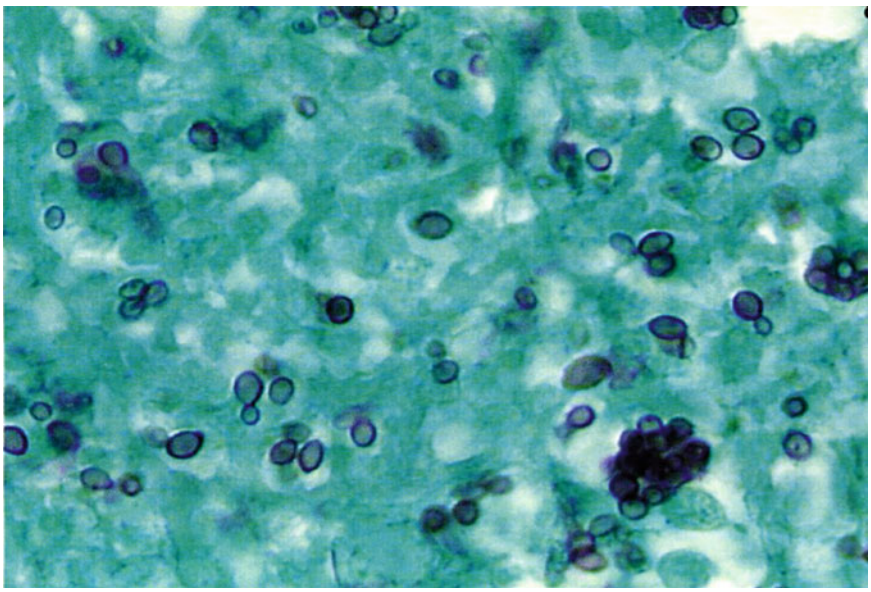

FIGURE 41-3. Liver biopsy in a transplant recipient who had fever, fatigue, elevated alkaline phosphatase, and pancytopenia. Gomori methenamine silver stain shows 2–4 µm oval budding yeasts typical of *H. capsulatum*.

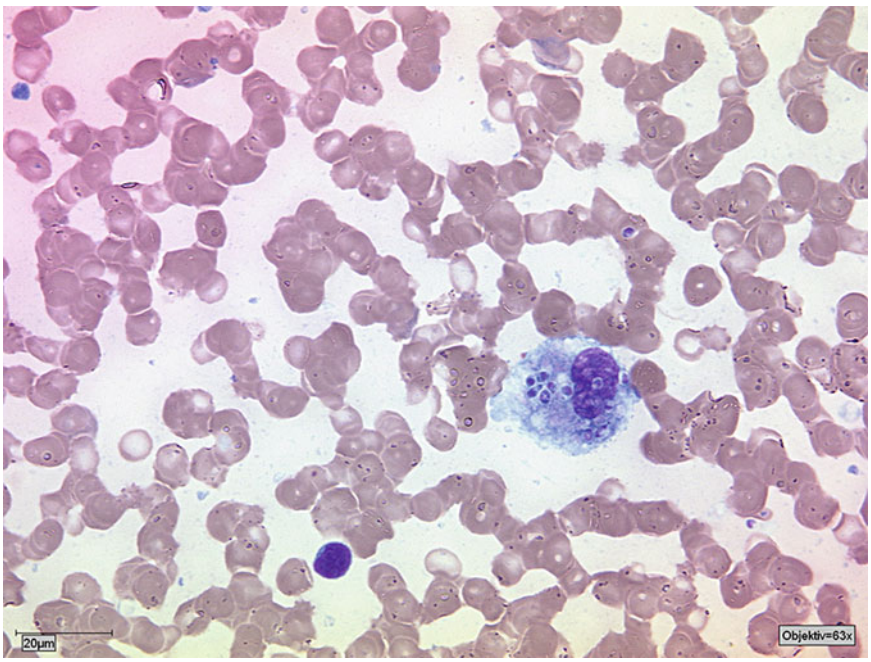

FIGURE 41-4. Peripheral smear showing *H. capsulatum* yeast forms inside a monocyte. The patient was acutely ill with high fevers, pancytopenia, and dyspnea.

False positive reactions with the Platelia *Aspergillus* galactomannan assay have been reported in immunosuppressed patients who have disseminated histoplasmosis, most likely because of the huge burden of circulating *Histoplasma* antigen in these patients [32, 44]. The converse is not the case: false positive *Histoplasma* antigen tests have not been reported in patients with invasive aspergillosis.

In a transplant recipient who has severe disease, biopsy of tissue to identify *H. capsulatum* is an important rapid diagnostic tool. Routine hematoxylin and eosin staining often will not reveal the small yeasts; either periodic acid–Schiff (PAS) stain or methenamine silver stain should be used (Figure 41-3). Appropriate tissues for sampling include the lung, mucocutaneous lesions, bone marrow, and liver. The organisms appear as small, 2–4-µm, oval, and budding intracellular yeasts. The organisms can sometimes be seen within neutrophils or monocytes on a peripheral blood smear (Figure 41-4).

41.2.5 Treatment

The Infectious Diseases Society of America guidelines for the treatment of histoplasmosis recommend antifungal treatment for all immunosuppressed hosts with histoplasmosis [45]. For patients who have moderate to severe pulmonary

histoplasmosis or who have disseminated histoplasmosis, liposomal amphotericin B, 3–5 mg/kg daily, is preferred. This formulation has been shown to have increased efficacy and decreased toxicity when compared with amphotericin B deoxycholate in patients with disseminated histoplasmosis [46]. After the patient shows clinical improvement, therapy can be changed to an azole to continue the course of therapy (Table 41-1).

The preferred azole for the treatment of histoplasmosis is itraconazole [45]. This is used for step-down therapy after

TABLE 41-1. Treatment of endemic mycoses in solid organ transplant recipients

	First line therapy	Alternative therapy	Comment
Histoplasmosis			
Mild localized acute pulmonary infection	ITRA loading dose as below[a], then 200 mg twice daily for at least 12 weeks	FLU 400–800 mg daily is a less preferred alternative	
Moderate to severe pulmonary infection	Liposomal AmB 3–5 mg/kg daily for 1–2 weeks until clinical improvement, followed by ITRA loading dose as below[a], then 200 mg twice daily for a total of 12 months	Alternatives for step-down therapy include VORI loading dose as below[b], then 200 mg twice daily, **OR** POSA loading dose as below[c], then 300 mg daily, **OR** FLU 400–800 mg daily, all for a total of 12 months	High-dose methylprednisolone should be considered for patients with severe hypoxemia or acute respiratory distress syndrome. Few patients have been treated with the alternative drugs Suppressive therapy with azoles should be considered in patients with persistent marked immunosuppression
Disseminated infection	Liposomal AmB 3–5 mg/kg daily for 1–2 weeks until clinical improvement, followed by ITRA loading dose as below[a], then 200 mg twice daily for a total of 12 months	Alternatives for step-down therapy include VORI loading dose as below[b], then 200 mg twice daily, **OR** POSA loading dose as below[c], then 300 mg daily, **OR** FLU 400–800 mg daily, all for a total of 12 months	Few patients have been treated with the alternative drugs Suppressive therapy with azoles should be considered in patients with persistent marked immunosuppression
Central nervous system infection	Liposomal AmB 5.0 mg/kg daily for 4–6 weeks, followed by ITRA loading dose as below[a], then 200 mg two or three times daily for a total of 12 months	Alternatives for step-down therapy include VORI loading dose as below[b], then 200 mg twice daily, **OR** POSA loading dose as below[c], then 300 mg daily, **OR** FLU 400–800 mg daily, all for a total of 12 months	Very few patients have been treated with the alternative drugs Suppressive therapy with azoles should be considered in patients with persistent marked immunosuppression
Blastomycosis			
Pulmonary infection	Liposomal AmB 3–5 mg/kg daily or ABLC 5 mg/kg daily for 1–2 weeks until clinical improvement noted, followed by ITRA loading dose as below[a], then 200 mg twice daily for a total of 12 months	Alternative for step-down therapy is VORI loading dose as below[b], then 200 mg twice daily for a total of 12 months. Less preferred alternatives are POSA, loading dose as below[c], then 300 mg daily, **OR** FLU 400–800 mg daily for a total of 12 months	Initial therapy with oral azoles is not recommended Suppressive therapy with azoles should be considered in patients with persistent marked immunosuppression
Disseminated infection	Liposomal AmB 3–5 mg/kg daily or ABLC 5 mg/kg daily for 1–2 weeks until clinical improvement noted, followed by ITRA loading dose as below[a], then 200 mg twice daily for a total of 12 months	Alternative for step-down therapy is VORI loading dose as below[b], then 200 mg twice daily for a total of 12 months. Less preferred alternatives are POSA, loading dose as below[c], then 300 mg daily, **OR** FLU 400–800 mg daily for a total of 12 months	Initial therapy with oral azoles is not recommended Suppressive therapy with azoles should be considered in patients with persistent marked immunosuppression
Central nervous system infection	Liposomal AmB 5 mg/kg daily for 4–6 weeks, followed by ITRA loading dose as below[a], then 200 mg twice daily for a total of 12 months	Alternatives for step-down therapy include VORI loading dose as below[b], then 200 mg twice daily **OR** FLU 400–800 mg daily for a total of 12 months. Less preferred is POSA loading dose as below[c], then 300 mg daily for a total of 12 months	Lifelong suppressive therapy with azoles should be considered

(continued)

Table 41-1. (continued)

	First line therapy	Alternative therapy	Comment
Coccidioidomycosis			
Mild localized acute pulmonary infection	FLU 200–400 mg/day **OR** ITRA loading dose as below[a], then 200 mg twice daily for 3–6 months		Monitoring at 1–3 months intervals is recommend for 1 year to determine resolution or relapse
Moderate to severe pulmonary infection	Liposomal AmB 3–5 mg/kg daily or ABLC 5 mg/kg daily for 1–2 weeks until clinical improvement noted, followed by ITRA loading dose as below[a], then 200 mg twice daily, **OR** FLU 400 mg once daily for a total of 12 months	Alternatives for step-down therapy include VORI loading dose as below[b], then 200 mg twice daily, **OR** POSA loading dose as below[c], then 300 mg daily for a total of 12 months	Lifelong suppressive therapy with azoles should be considered
Disseminated, non-meningeal infection	Liposomal AmB 3–5 mg/kg daily or ABLC 5 mg/kg daily for 1–2 weeks until clinical improvement noted, followed by ITRA loading dose as below[a], then 200 mg twice daily **OR** FLU 400 mg once daily for a total of 12 months	Alternatives for step-down therapy include VORI loading dose as below[b], then 200 mg twice daily, **OR** POSA loading dose as below[c], then 300 mg daily for a total of 12 months	Lifelong suppressive therapy with azoles should be considered
Central nervous system infection	FLU 800 mg daily given as lifelong therapy	VORI loading dose as below[b], then 200 mg twice daily **OR** ITRA loading dose as below[a], then 200 mg twice daily, **OR** possibly POSA loading dose as below[c], then 300 mg daily all given as lifelong therapy	If concomitant disseminated infection and central nervous system infection are present, intravenous liposomal AmB or ABLC should be given for systemic infection, as well as FLU for central nervous system infection

AmB amphotericin B, *ABLC* amphotericin B lipid complex, *FLU* fluconazole, *ITRA* itraconazole, *POSA* posaconazole, *VORI* voriconazole.

[a]Oral itraconazole loading dose is 200 mg three times daily for 3 days. Because of problems with absorption and drug–drug interactions with itraconazole, therapeutic drug monitoring is strongly recommended. Itraconazole serum levels should be determined after ~2 weeks of treatment. Random itraconazole serum concentrations of at least 1.0 μg/mL are suggested, but we prefer to have levels closer to 2 μg/mL.

[b]Oral voriconazole loading dose is 400 mg twice daily for the first day. Because of problems with absorption and drug–drug interactions with voriconazole, therapeutic drug monitoring is strongly recommended. Voriconazole serum trough levels should be determined after ~1 week of treatment. Target voriconazole serum trough levels should range between 1.0 and 5.5 μg/mL.

[c]Oral posaconazole delayed release tablets loading dose is 300 mg twice daily for the first day. Because of problems with absorption and drug–drug interactions with posaconazole, therapeutic drug monitoring is strongly recommended. Posaconazole serum levels should be obtained after 5–7 days of treatment, with a goal of >1.0 μg/mL.

amphotericin B and for initial therapy for the uncommon presentation of mild localized pulmonary histoplasmosis in a transplant recipient. The loading dose is 200 mg three times daily for 3 days, followed by 200 mg twice daily. Better absorption is seen with the oral solution than the capsules. The oral solution should be given on an empty stomach; the capsules are given with food, and medications that inhibit gastric acid secretion must be avoided. The total length of therapy should be 12 months for most transplant recipients. Because of issues with absorption and many drug–drug interactions, it is imperative to measure serum concentrations of itraconazole to be sure that adequate levels are attained (Table 41-1).

Fluconazole should be considered a second-line agent for histoplasmosis; the response to primary therapy is less and the relapse rates are higher when it is compared with itraconazole, especially in an immunosuppressed population [45]. If a patient cannot tolerate itraconazole because of side effects or poor absorption, fluconazole, 800 mg daily, could be used, but the newer azoles, voriconazole and posaconazole, are increasingly used in this circumstance.

There are no clinical trials studying voriconazole or posaconazole for treatment of histoplasmosis. However, both agents are active against *H. capsulatum* in vitro. Increasingly, patients are reported who have been treated successfully with voriconazole, usually as step-down therapy after amphotericin B [16, 17, 24, 47]. Posaconazole has been reported as showing efficacy in patients with histoplasmosis, but none of these patients were transplant recipients [48, 49]. Isavuconazole, the newest azole agent, appears to have in vitro activity against *H. capsulatum*. However, only seven patients with histoplasmosis were treated in a salvage trial using isavuconazole. Although success was noted, there were too few patients to suggest that isavuconazole should be used to treat histoplasmosis. Echinocandins are not active and should not be used for the treatment of histoplasmosis.

Whether transplant recipients should be maintained on long-term suppressive therapy to prevent relapse of histoplasmosis has not been established. Reports of relapses after amphotericin B or azole treatment was stopped have been published [16, 17]. It seems prudent to continue therapy with itraconazole at dosages of 200–400 mg daily as long as the

patient is markedly immunosuppressed, but there are no clinical trials, as there are with AIDS patients, on which to base this practice. The decision about the use of long-term azole therapy should be based on the clinician's appraisal of the extent of immunosuppression and the possibility of recovery of cell-mediated immunity.

41.3 Blastomycosis

41.3.1 Epidemiology

The endemic area for *B. dermatitidis* includes the southeastern, south central, and north central United States, the Canadian provinces of Ontario, Manitoba, and Saskatchewan, and focal geographic areas in Africa and Europe. The primary habitat of the organism is likely soil and decaying vegetation, especially in close proximity to waterways [50, 51]. Infection occurs predominantly in middle-aged men, many of whom have outdoor occupations and hobbies. Blastomycosis is the least commonly reported endemic mycosis in the USA. Although small outbreaks have occurred, large epidemics, as have occurred with histoplasmosis and coccidioidomycosis, have not been reported.

This relatively uncommon mycosis has been reported rarely in transplant recipients. Cases have occurred more often in solid organ transplant recipients than in hematopoietic stem cell transplant recipients [3, 8, 18, 24, 35, 52–56]. Patients who have received a stem cell transplant are often not well enough while they are on immunosuppressive agents to indulge in activities that might expose them to *B. dermatitidis*. Blastomycosis has been reported in solid organ transplant recipients years after transplantation when they are back at work and doing outdoor activities, but still on immunosuppressive therapy [8]. Most stem cell transplant recipients, unless they develop graft-versus-host disease, are off immunosuppressive therapy by this time and perhaps no longer in a high-risk category.

41.3.2 Pathogenesis

Blastomycosis is primarily a pulmonary infection; infection is initiated when the conidia are inhaled from the environment. Rare cases of inoculation blastomycosis, one of which occurred in a renal transplant recipient, have been described [55]. Dissemination to the skin, osteoarticular structures, and genitourinary tract is common. A common presentation in healthy hosts is the development of single or multiple skin lesions at a time when the pulmonary focus has already resolved. Reactivation occurring years after the initial exposure has been documented, but this appears to occur less often than with histoplasmosis [53, 57]. No cases of blastomycosis transmitted by a donor organ have been documented.

Neutrophils and macrophages, as well as T lymphocytes, appear to be important components of the host response to infection with *B. dermatitidis* [50]. This dual response may be another reason that blastomycosis, when compared with histoplasmosis, is not common among transplant recipients.

41.3.3 Clinical Manifestations

Transplant recipients appear more likely than healthy hosts to develop severe pulmonary or disseminated blastomycosis [3, 8, 18, 24]. However, even when symptomatic dissemination occurs, it mimics the situation in healthy hosts in that the major target organ is the skin rather than the viscera. Among transplant recipients, the cutaneous lesions tend to be pustular (Figure 41-5a) and ulcerative and less often the typical verrucous lesions usually seen with blastomycosis (Figure 41-5a). The types of pulmonary lesions in transplant recipients are similar to those seen in healthy hosts, and they include lobar pneumonia, cavitary pulmonary lesions, and diffuse infiltrates [8, 18, 24]. Adult respiratory distress syndrome may be more common in immunosuppressed patients, including transplant recipients, and is associated with a high mortality rate [58] (Figure 41-6). Central nervous system

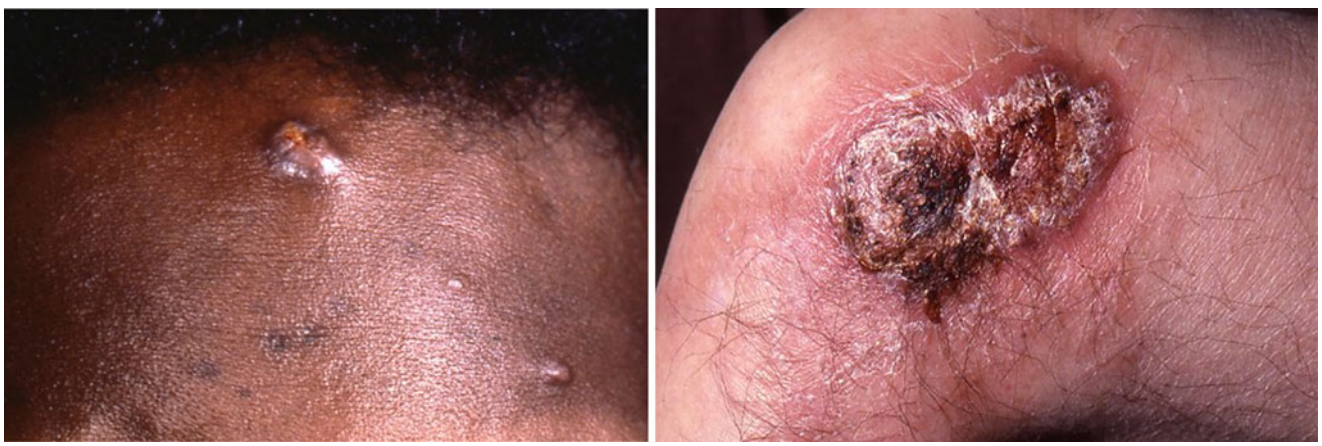

FIGURE 41-5. (**a**) Pustular skin lesions that appeared over a few days in an immunosuppressed patient and that yielded *B. dermatitidis* in culture. (**b**) More typical verrucous skin lesion of blastomycosis in an older man.

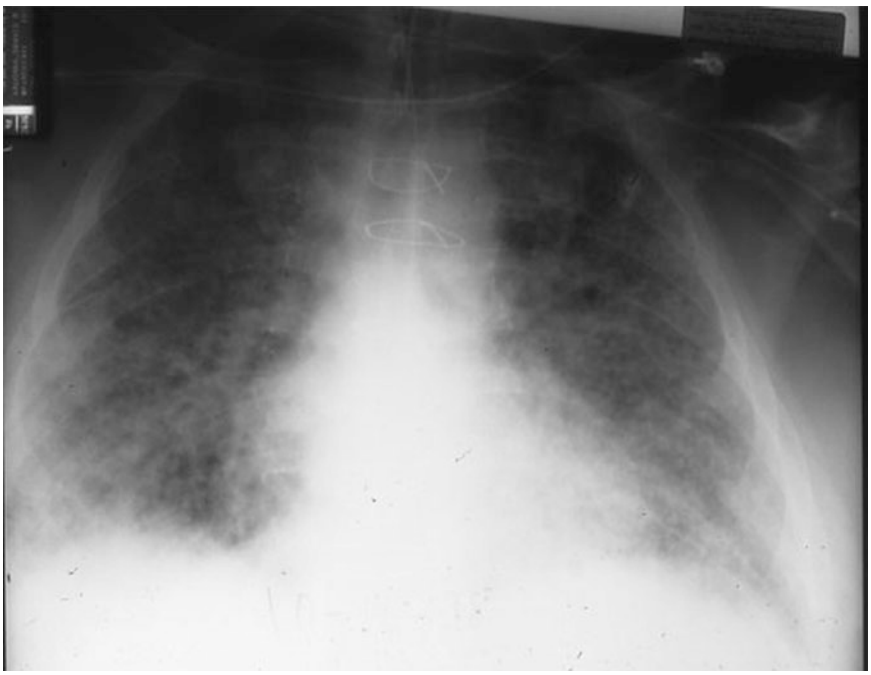

FIGURE 41-6. Chest radiograph from a patient who had acute respiratory distress syndrome (ARDS) due to *B. dermatitidis.*

infection with either meningitis or intracerebral mass lesions is a rare manifestation of blastomycosis that can occur in transplant recipients [3, 18].

41.3.4 Diagnosis

The diagnosis of blastomycosis is best made by culture of the organism from sputum, bronchoalveolar lavage fluid, urine, skin, or visceral lesions. However, histopathologic examination of a skin lesion and cytological examination of sputum or bronchoalveolar lavage fluid are reasonably sensitive and specific and are much quicker than culture methods that can take as long as several weeks to reveal growth of the mycelial form of *B. dermatitidis* [50, 54, 59]. When pustular skin lesions are present, a simple potassium hydroxide smear of purulent material aspirated from the lesion can quickly reveal the organism. Lung or other tissue biopsy specimens show the yeasts when periodic acid–Schiff or methenamine silver stains are performed. In tissues, *B. dermatitidis* appear as distinctive large (5–20 μm), thick-walled yeasts that retain a broad-based connection to the budding daughter cell (Figure 41-7).

The standard serological assays (complement fixation and immunodiffusion) for blastomycosis are neither sensitive nor specific. An enzyme immunoassay that detects the galactomannan cell wall antigen of *B. dermatitidis* is increasingly used and appears to be a useful diagnostic tool [24, 54, 60, 61]. The assay is performed on urine and serum. The major drawback is that is not specific for *B. dermatitidis*, and cross reactivity is almost routinely noted with *H. capsulatum*.

41.3.5 Treatment

The Infectious Diseases Society of America guidelines for the treatment of blastomycosis recommend that transplant recipients and other immunosuppressed patients who acquire blastomycosis should be treated initially with an amphotericin B formulation [62]. Relapses and failures have been documented in those transplant recipients who were treated with an azole as initial therapy [18, 24]. Lipid formulations of amphotericin B should be used in transplant recipients, who are often on other nephrotoxic drugs. The dosage is 3–5 mg/kg daily of liposomal amphotericin B or 5 mg/kg daily of amphotericin B lipid complex. After the patient shows a good clinical response, therapy can be changed to oral itraconazole, for a total of 12 months of antifungal therapy. The loading dose of itraconazole is 200 mg three times daily for 3 days, followed by 200 mg twice daily. Better absorption is seen with the oral solution than the capsules. The oral solution should be given on an empty stomach, the capsules are given with food, and medications that inhibit gastric acid secretion must be avoided (Table 41-1).

If the patient cannot tolerate itraconazole, voriconazole is the second-line choice. Experience is limited in the transplant population [3, 8, 47, 63]. Voriconazole has been reported to successfully cure CNS blastomycosis in individual case reports and small series of cases [47, 63–66]. A multi-center review of 22 cases of CNS blastomycosis noted success in 9 of 10 patients who had been treated with voriconazole, and recommended voriconazole as the step-down agent of choice for this uncommon complication of blastomycosis [63]. Fluconazole is not as effective as itraconazole

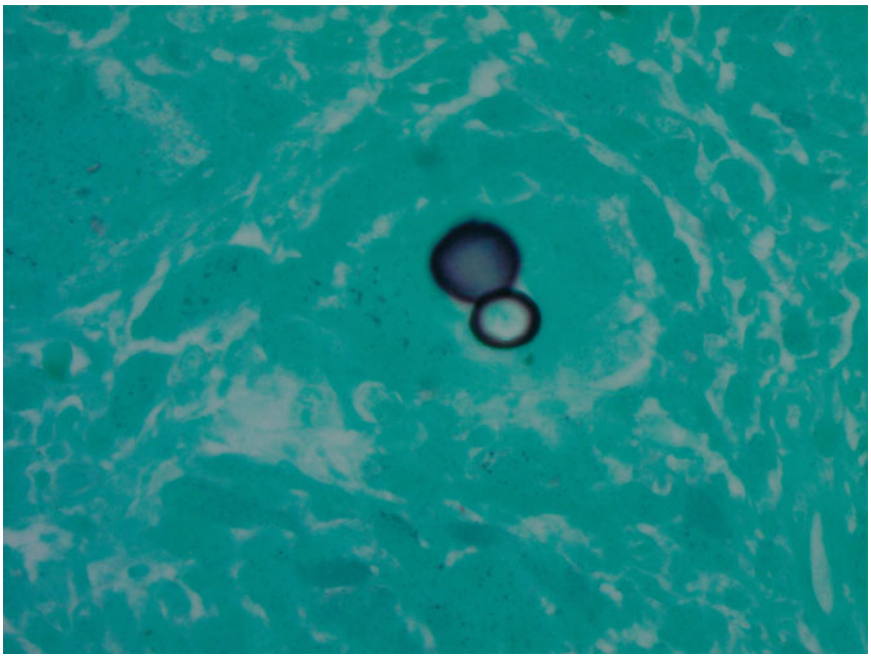

FIGURE 41-7. Lung biopsy sample showing a large, thick-walled, yeast showing broad-based budding, which is typical of *B. dermatitidis.*

for blastomycosis, and there is little experience treating blastomycosis with posaconazole [3, 67]. Echinocandins are not active and should not be used for the treatment of blastomycosis. Long-term suppressive therapy with itraconazole, 200–400 mg daily, may be prudent in transplant recipients who remain on immunosuppressive agents. The need will depend on the clinician's appraisal of the patient's immune status.

41.4 Coccidioidomycosis

41.4.1 Epidemiology

There are now two recognized species of *Coccidioides*, *C. immitis*, which is endemic in southern California, and *C. posadasii,* which is endemic in Arizona, parts of New Mexico and western Texas, and Central and South America. The clinical picture is identical and most laboratories cannot differentiate the two. *Coccidioides* species are restricted mostly to the arid conditions typifying the Lower Sonoran Life Zone, but in times of exuberant growth, the conidia can be swept into more distant areas. Recent reports describe endemic foci of *Coccidioides* species in arid areas of Washington and Utah [68, 69]. Infection is very common because of the propensity of the mycelial form of the organism to be easily dispersed [4, 70]. Most of the population in the endemic area becomes infected before adulthood. For reasons still unknown, dark-skinned persons, especially African Americans, are less likely to contain the organism during primary infection and more likely to develop disseminated coccidioidomycosis [5].

In transplant recipients, coccidioidomycosis is more prevalent than blastomycosis but less common than histoplasmosis [1–3]. Most transplant recipients reported to have developed coccidioidomycosis had received a solid organ transplant [7, 11–15, 19, 71–74]. Fewer patients have been reported who developed coccidioidomycosis after receiving a hematopoietic stem cell transplant [3, 35, 75–77].

41.4.2 Pathogenesis

The arthroconidia produced by *Coccidioides* species are easily dispersed by desert winds. After inhalation into the lungs, the conidia undergo a morphologic change, becoming large (80–100 μm), thick-walled spherules that become stuffed with enormous numbers of endospores. When the spherule is filled, it ruptures, releasing the endospores; each endospore has the potential to form a new spherule, thus perpetuating the infection. Most persons who develop coccidioidomycosis likely have asymptomatic dissemination to multiple organs, but the host with an intact immune system may experience only symptoms of a mild respiratory illness.

The primary host defense against *Coccidioides* species appears to be cell-mediated immunity. Although neutrophils are present in most coccidioidal lesions, they are unable to eradicate spherules [5]. Solid organ transplant recipients who remain on immunosuppressive agents for an extended period remain at risk of developing coccidioidomycosis [19].

In addition to infection that is related to a new exposure to *Coccidioides* species, reactivation coccidioidomycosis has been described in transplant recipients [19, 73, 77]. Reactivation is most likely to occur when a decrease in

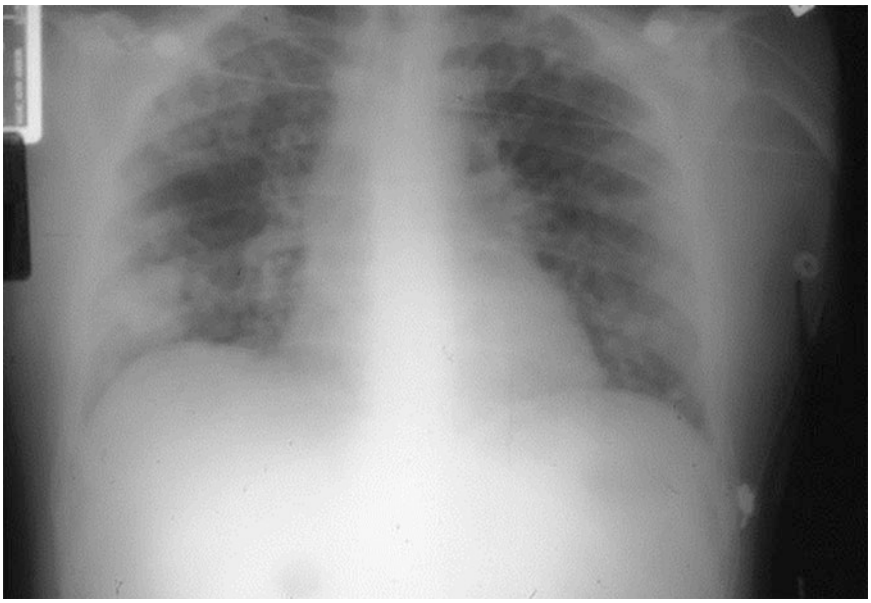

FIGURE 41-8. Chest radiograph of an immunosuppressed patient who ran a landscaping company in Tucson, Arizona.

cell-mediated immunity happens, such as occurs at the time of transplantation. Differentiating new infection from reactivation is difficult among patients living in the endemic area. Approximately half of all cases of coccidioidomycosis are estimated to occur within the first 3 months after solid organ transplantation, and these are likely due to reactivation [19]. Not unexpectedly for an organism that can survive in a latent state for years, *Coccidioides* species have been transmitted with the donor organ [3, 11–13]. The few instances in which this occurred were marked by rapid onset of infection post-transplantation and death due to overwhelming coccidioidomycosis.

The outcome of coccidioidomycosis in transplant recipients appears to be improving, but mortality still remains high. Early mortality rates in kidney transplant recipients were reported to be as high as 63% [81], but recently the mortality has been reported to be approximately 25% [19, 82]. Mortality rates among liver transplant recipients have been reported to be higher than those noted in kidney transplant recipients [71, 73], and heart transplant recipients appear to have the lowest mortality rates from coccidioidomycosis [74], but the number of cases in these latter groups is small. In the largest reported series of coccidioidomycosis in allogeneic stem cell transplant recipients, the mortality was 45% [77].

41.4.3 Clinical Manifestations

Acute pulmonary coccidioidomycosis in the healthy host is generally a self-limited infection. In hosts who are immunosuppressed, severe pneumonia with high fever, hypoxemia, and diffuse infiltrates is more likely to occur (Figure 41-8). However, a wide spectrum of pulmonary manifestations that includes nodular lesions, diffuse interstitial infiltrates, alveolar infiltrates, and cavitary lesions has been described [19, 77, 78].

In transplant recipients, *Coccidioides* is likely to disseminate widely. The sites most often involved are the skin, bones, subcutaneous tissues, and meninges. Fungemia has been documented in individual patients and appears to be more common among transplant recipients [12, 72, 73, 79, 80]. The skin lesions are typically papular, pustular, or nodular, and they often ulcerate. The symptoms of meningeal involvement include headache, cranial nerve palsies, and signs of increased intracranial pressure. In the transplant recipient, meningeal involvement is usually associated with widespread disseminated infection.

41.4.4 Diagnosis

The definitive diagnostic test for coccidioidomycosis is growth of the organism. *Coccidioides* species are the most easily grown of the endemic mycoses. Appropriate material for culture includes tissue biopsies, aspirated material from subcutaneous lesions, synovial fluid, sputum, bronchoalveolar lavage fluid, cerebrospinal fluid, and blood. In the laboratory, *Coccidioides* grows as a mold on most routine laboratory media within 3–7 days. Because the mycelial phase is highly contagious, laboratory workers should always be notified that coccidioidomycosis is a diagnostic consideration so that they can take the necessary precautions to protect themselves from exposure. Because *Coccidioides* species are considered to be bioterrorism agents, a laboratory suspecting that a culture contains *Coccidioides* species is required by law in the USA to seal the culture and send it immediately to a reference laboratory for identification.

The identification of *Coccidioides* in tissues and body fluids can be carried out while awaiting culture results. This is

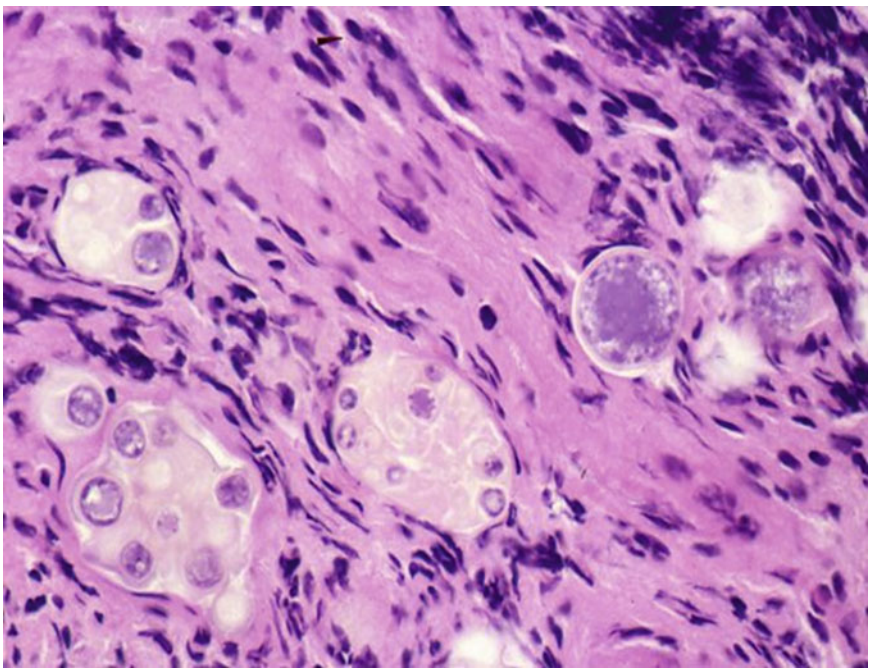

FIGURE 41-9. Lung biopsy from the patient whose chest radiograph is shown in Figure 41-8, showing several large spherules of *C. posadasii.*

especially important in a patient who is immunosuppressed and who is extremely ill with pulmonary or disseminated coccidioidomycosis. Spherules can be visualized in potassium hydroxide or calcofluor preparations of sputum or material aspirated from a lesion and also in cytological preparations from body fluids or bronchoalveolar lavage fluid. In tissue biopsies, the spherules are large enough (80–100 μm) to be seen with routine hematoxylin and eosin stains, and they also can be sought using silver methenamine staining. The spherules are quite distinctive and are easily identifiable (Figure 41-9).

For immunocompetent patients with coccidioidomycosis, serology is a useful diagnostic tool. However, in immunosuppressed patients who have disseminated infection, the serologic assays can be negative [83]. For patients with suspected coccidioidomycosis, serologic testing should be performed at a reference laboratory that has expertise in assays for *Coccidioides.* An assay for *Coccidioides* antigen has become available, but it is unclear whether this will prove useful for transplant recipients who have coccidioidomycosis [84].

41.4.5 Treatment

All transplant recipients with coccidioidomycosis should be treated with an antifungal agent [85]. For all but the most benign pulmonary infections, a lipid formulation of amphotericin B, 3–5 mg/kg daily, is recommended. After the patient shows a clinical response, treatment can be changed to an oral azole, either itraconazole, 200 mg three times daily for 3 days then 200 mg twice daily, or fluconazole, 800 mg for one dose then 400 mg once daily [86] (Table 41-1). Treatment usually is given for at least 12 months, and for transplant patients who remain immunosuppressed, lifelong therapy may be needed [19], as relapse after the cessation of therapy has been described [72, 74, 81]. It seems prudent to use suppressive azole therapy, either fluconazole or itraconazole, especially in those patients who have had disseminated infection and in those at higher risk for severe coccidioidomycosis, such as African Americans.

Several small series have reported on the use of posaconazole for the treatment of coccidioidomycosis [87–90]. To date, the experience in the transplant population remains limited. Voriconazole also has activity against *Coccidioides* species, and has been shown to be efficacious in individual patients, including those who have meningitis [91, 92]. Kim et al retrospectively compared their experience with voriconazole and posaconazole in 37 patients with coccidioidomycosis, 5 of whom were transplant recipients. Success rates were similar between the two agents; with voriconazole, 67% showed improvement, and with posaconazole, 75% were improved [90]. Isavuconazole has been used in a small number of patients with coccidioidomycosis with some success, but the numbers are too small to recommend this agent as an alternative therapy at this time. Echinocandins should not be used for the treatment of coccidioidomycosis.

Fluconazole is the agent of choice for coccidioidal meningitis [85]. In the transplant recipient who has meningitis as one manifestation of widespread dissemination, lipid amphotericin B should be used to treat the systemic infection, in addition to fluconazole for treatment of meningitis. The recommended daily dose of fluconazole for treating

meningitis is 800 mg. The intrathecal administration of amphotericin B deoxycholate has been a standard treatment for coccidioidal meningitis for many years, but because of the difficulty of administration and the high incidence of severe side effects, is used only when azole therapy has failed. All patients with coccidioidal meningitis require lifelong suppressive therapy, usually with fluconazole, to prevent relapse.

Several transplant centers within the endemic area use serology to routinely screen prospective transplant recipients and donors for evidence of prior infection with *Coccidioides* species [19, 77, 93]. Solid organ transplant recipients who have antibodies or a prior history of coccidioidomycosis within the preceding 1–2 years are routinely given prophylaxis with fluconazole, 400 mg daily for the first year post-transplantation, and 200–400 mg thereafter [93]. If active coccidioidomycosis or antibodies are found in the donor, the recipient is given fluconazole prophylaxis for life [93]. It has been recommended that patients undergoing allogeneic stem cell transplantation who have had a history of coccidioidomycosis or who have antibodies to *Coccidioides* on serological testing should be given fluconazole prophylaxis until full recovery of T cell function can be documented [77] (Table 41-1).

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Part VII
Other Infections

42
Toxoplasmosis After Hematopoietic Stem Cell Transplantation

Rodrigo Martino

Toxoplasma gondii is a protozoan that commonly infects animals and birds. Although *T. gondii* infection in humans is usually asymptomatic, clinical disease occurs in the immune suppressed patient. Infection may be acute (recently acquired) or chronic (latent). *T. gondii* exists in three forms during its life cycle: the tachyzoite, which is the asexual invasive form; the tissue cyst (containing bradyzoites), which persists in the tissues of the infected host during the chronic phase of the infection; and the oocyst (containing sporozoites), which is produced during the sexual cycle in the intestine of the definitive host—the cat. Transmission to humans occurs by ingesting tissue cysts or oocysts, or by blood product transfusion or organ transplantation. Following infection by oral ingestion, tachyzoites disseminate from the gastrointestinal tract and can invade virtually any cell or tissue where they proliferate and produce necrotic foci surrounded by inflammation. In immune suppressed patients, acute infection may result in severe damage to multiple organs. Even in individuals with a normal immune response, tissue cysts form in multiple organs (latent infection), and can subsequently give rise to a severe localized reactivation producing, for example, toxoplasma encephalitis or chorioretinitis.

Toxoplasmosis appears to be a relatively rare opportunistic infection following hematopoietic stem cell transplantation (HSCT). Up to the late 1990s, only 55 cases had been reported after HSCT [1–3], which contrasted with the high frequency of this complication in other patient populations with severe cellular immunodeficiencies, mainly advanced acquired immunodeficiency syndrome (AIDS). Table 42-1 summarizes the case series of toxoplasmosis in HSCT published until mid-2015, and all other cases have been described as case reports. As of mid-2015, around 300 cases of toxoplasmosis have now been reported in HSCT in peer-reviewed manuscripts, a figure that still seems small when compared with other relevant infectious complications in these patients.

The seroprevalence for *T. gondii* varies greatly between and even within countries, ranging from <15% in some North American and Japanese studies [4] and in pediatric wards, to 50–80% of adult HSCT recipients in countries with high endemicity such as France or Turkey [1, 5, 16]. This varying seroprevalence is probably the main reason for the great variability in the frequency of diagnosed cases of toxoplasmosis after HSCT, which has been estimated to average 0.8% [17], with <0.4% in areas of low endemicity to 2–3% in those with high antibody prevalence. The disease, however, is underdiagnosed, since more than half of the cases reported in the literature were diagnosed at autopsy (see Table 42-1).

Toxoplasmosis occurs mainly in allogeneic transplant recipients, although cases after autologous transplants have been published [2, 7, 18], and some are included in Table 42-1. Around 90% of patients are seropositive before HSCT, indicating that reactivation of latent tissue cysts in previously infected individuals is the usual mechanism implicated, as has been demonstrated in AIDS patients. It is, thus, important to determine the patients' serostatus prior to transplant. However, the disease may also develop in seronegative recipients from seronegative donors, suggesting that primary infection after transplant may also occur, and primary infections may be more severe than reactivations [46]. The disease usually begins early after transplant with 95% of the cases occurring within the first 6 months after the procedure, although late cases may occur, usually in patients with chronic graft-versus host disease (GVHD) requiring immunosuppressive treatment [7, 19]. Acute GVHD has been suggested as a possible predisposing factor for Toxoplasma disease, and in a study by the European Group for Blood and Marrow Transplantation (EBMT) [7] 77% of cases occurred in patients with moderate-to-severe acute GVHD or chronic GVHD. The central nervous system (CNS) is the main site of disease, but pneumonitis and myocarditis are also frequent findings, particularly when the diagnosis is made at autopsy. In fact, myocarditis, nephritis, and involvement of other deep organs are rarely made clinically but are frequent findings at autopsy [4, 7, 20].

Several recent patient series have added further insight into the importance of not overlooking this infection in this patient population (Table 42-1). A study from the Memorial Sloan-Kettering Cancer Center in New York described ten cases of disseminated toxoplasmosis among 463 patients

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TABLE 42-1. Results of published case series of toxoplasmosis after HSCT

Author (references)	No. of cases	Number of transplants (% frequency)	Percentage of seropositivity pretransplant in the entire transplant cohort	No. of patients seropositive pretransplant	Median day onset posttransplant (range)	No. treated for toxoplasmosls	No. survived toxoplasmosls	Comments
Derouin et al. [1]	7	296 AlloBMT (2.4)	NS	7	74 (55–180)	2	2	
Slavin et al. [4]	12	3803 AlloBMT (0.31), 509 autoBMT (0)	15	11/11 tested	59 (35–97)	NS	2	
Bretagne et al. [5]	2	550 AlloBMT (0.3)	70	NS	NS	NS	NS	
Chandrasekar et al. [2]	3	662 (0.5)	NS	NS	46 (1–90)	1	None	
Maschke et al. [6]	20[a]	655 (3.1)	NS	20/20	73 (14–689)	NS	5	
Martino et al. [7]	41	4391 AlloHSCT (0.93), 7097 autoHSCT (0)	Variable	31/33 tested	64 (4–516)	23	14	Late disease (after day +63) and having received therapy were associated with improved survival
Mele et al. [8]	2	631 AlloHSCT	NS	2/2	30, 169	1/2	1/2	
Roemer et al. [9]	8	301 AlloHSCT	NS	8/8	120 (41–280)	4/8	3	CD4+ cell counts <100/μL
Small et al. [10]	10	463 AlloBMT (2.2)	23	7/10	78 (36–155)	5	1	Risk factors for toxoplasmosis were: unrelated donor BMT and recipient seropositivity pretransplant
Matsuo et al. [11]	2	925 AlloHSCT (0.2), 641 autoHSCT (0)	NS	NS	60, 100	2	2	Seroprevalence NS
Janitschke et al. [12]	3	75 AlloBMT (4)	71	3/3	72 (38–135)	3	1	18/22 Seronegative recipients became IgG positive shortly after transplant
Lim et al. [13]	2	220 AlloHSCT (0.9)	30	2/2	45, 95	2	1	All patients received in vivo T-cell depletion with alemtuzumab
Aoun et al. [14]	7	121 AlloHSCT (5), 204 autoHSCT (0.4)	69	4/7	45 (13–140)	7	6	The authors suggest first line therapy with pyrimetha mine clindamycin
de Medeiros et al. [15]	9	789 AlloHSCT (1.14)	NS	9/9	69 (13–265)	1	1	8 cases diagnosed only at autopsy

Rusinakova et al. [35]	4	116 AlloHSCT (2.6%) 395 AutoHSCT (0.3%)	NS	4/4	45 (20–75)	3	3	1 case diagnosed at autopsy only
Mulanovich et al. [36]	9	3626 AlloHSCT (0.25) US pts 0.15 Non-US pts 1.6%	18% US pts >50% Non-US pts	7/7	56 (12–122)	7	1	2 cases diagnosed only at autopsy
Bautista [37]; Martino et al. [38] (cases in adult CBT)	9	148 adult CBT (4%)	45%	6/9	39 (7–98)	7	2	2 cases diagnosed only at autopsy Prophylaxis had been given only to 1/9 cases
Busemann et al. [39]	3	155 AlloHSCT (1.8%)	40%	3/3	75 (32–395)	1	0	2 cases diagnosed only at autopsy None had received prophylaxis
Sumi et al. [40]	6	5/279 AlloHSCT (1.8%) 1/87 AutoHSCT (1.1%)	10%	5/5	NS	2	1	4 cases diagnosed only at autopsy
Hakko et al. [41]	5	170 AlloHSCT (2.9%)	70%	5/5	42 (26–119)	5	2	Study focused only on CNS toxoplasmosis
Nigro et al. [42]	4	12 AlloHSCT (33%)	100%	4/4	25 (18–59)	2	2	2 cases diagnosed only at autopsy, with negative results of the qualitative PCR used for monitoring

Abbreviations: *BMT* bone marrow transplantation, *AlloHSCT* allogeneic hematopoietic stem cell transplantation, *autoHSCT* autologous HSCT, *NS* not specified, *pts* patients, *CBT* cord blood transplantation.
[a]4 definite and 16 possible cases of toxoplasmosis.

who received T-cell-depleted allogeneic bone marrow transplantation (2.2% frequency) [10]. When compared with other studies this frequency appears to be high, especially when considering that the pretransplant seroprevalence was only 23% and that these patients had a very low incidence of moderate-to-severe GVHD. This experience suggests that T-cell depletion may be an independent risk factor for this infection, although a case-control study would be needed to confirm this suspicion. Two other studies have been recently published by the EBMT Infectious Diseases Working Party [7, 21]. The first study summarized the results of a survey among European transplant centers, which showed that this infection occurs almost exclusively after an allogeneic HSCT, with 41 cases diagnosed after 4391 allogeneic HSCT (frequency 0.93%) and none after 7097 autologous HSCT [21]. However, as previously stated, cases have been described after autologous HSCT. Additionally, we have recently seen a case of pulmonary toxoplasmosis 10 months after a CD34+-cell selected autologous HSCT, suggesting that T-cell depletion may also increase the risk after autologous transplants.

Toxoplasma encephalitis typically presents with focal neurologic abnormalities of subacute onset, frequently accompanied by nonfocal signs and symptoms such as headache, altered mental status, and fever. The most common focal neurologic sign is motor weakness, but patients may also present with cranial nerve abnormalities, speech disturbances, visual field defects, sensory disturbances, cerebellar signs, focal seizures, and movement disorders. Meningeal signs are very rare. The cerebral spinal fluid may show slight mononuclear pleocytosis, increased protein, and normal glucose levels. Computed tomography (CT) brain scans often show multiple bilateral cerebral lesions that tend to be located at the corticomedullary junction and the basal ganglia. These lesions are generally hypodense and show ring enhancement after intravenous contrast injection. Magnetic resonance imaging (MRI) scans show lesions as high signal abnormalities on T2-weighted imaging, although other nonspecific space-occupying lesions may be seen [6, 7]. MRI is more sensitive than CT in the early diagnosis of this infection [6].

Toxoplasma pneumonitis may develop in the absence of extrapulmonary disease. Its clinical and radiologic features are nonspecific and may mimic interstitial pneumonitis due to other causes [22–24].

Toxoplasma chorioretinitis appears surprisingly rare compared to the incidence noted in the AIDS population, particularly since many transplant programs utilize eye examination routinely pre- and posttransplant because of the incidence of chronic ocular GVHD posttransplant [25]. Interestingly, two cases of reactivation of toxoplasma chorioretinitis were reported in recipients of autologous transplants [26].

Since toxoplasmosis is so difficult to diagnose histologically in these patients, noninvasive diagnostic tests would be of utmost importance. Isolation of the parasite from blood or body fluids using rodents or cell culture techniques is time-consuming, expensive, and is available only in few routine microbiology laboratories. In the HSCT recipient, the utility of serology is mainly to identify those at risk for developing toxoplasmosis posttransplant, since serologic studies posttransplant are seldom of use. Polymerase chain reaction (PCR) techniques were developed for diagnosis of neonatal infections and for the noninvasive diagnosis of cerebral toxoplasmosis in patients with AIDS [27]. These techniques are applicable in blood, cerebrospinal fluid (CSF), and bronchoalveolar lavage (BAL); the usual samples that are available in HSCT recipients with this infection. However, PCR techniques are not standardized, and thus the results of published studies are difficult to interpret. In AIDS, patients with brain lesions PCR in blood and CSF have a sensitivity of 50–65% and a specificity of 95–100% for toxoplasmosis [28]. Currently, however, the predictive values of any PCR technique for infectious agents in AIDS, HSCT recipients, and other patient populations depend mainly on the type of PCR and laboratory protocols used. However, many centers have developed and use a quantitative PCR with a level of detection as low as 20 parasites/mL, with parasite loads of >600/mL reported in most patients with toxoplasmosis [29]. In the EBMT study 46% of the patients with *Toxoplasma* disease and all six with infection had at least one positive PCR result, thus confirming the widespread use of this diagnostic technology in clinical practice [7]. Particularly interesting are the patients with positive PCR tests from blood samples without evidence of disease [30]. These patients may represent a transitional state between the local reactivation of tissue cysts into tachyzoites and the establishment of localized or disseminated tissue destruction by replicating tachyzoites favored by the intense cellular immunosuppression after transplant or during GVHD. This observation would be somehow similar to the early detection of cytomegalovirus (CMV) infection by PCR or the pp65 antigenemia test. Unlike the latter, however, the clinical significance of detecting *T. gondii* DNAemia is currently unknown. On the other hand, several cases of well-documented disseminated toxoplasmosis with negative serum PCR results have been described [20], and our patient with pulmonary toxoplasmosis described earlier had a negative PCR in blood samples but positive cytology and PCR in BAL samples. The earlier onset of *Toxoplasma* infection (median day 35, range 13–51) than disease (median day 64, range 4–516) in the EBMT study suggests that infection may indeed precede disease in many cases [7]. Thus, research efforts to establish the role of PCR in this setting are clearly warranted. Unfortunately, as with other PCR-based diagnostic tests for infectious diseases, the technique is not standardized, making comparisons between centers difficult unless a quality control is established [29]. Table 42-2 summarizes the published studies that analyze the potential role of screening peripheral blood (PB) samples for the early diagnosis and/or preemptive therapy of toxoplasmosis as of mid-2015. These studies emphasize the

TABLE 42-2. Results of published studies that analyze the role of monitoring PB samples with a PCR for *Toxoplasma gondii* after HSCT

Author (references)	Number of patients	Percentage of seropositivity pretransplant (%)	Number of (percentage) toxoplasma infections in seropositive patients (PCR+)	Number (percentage) toxoplasma disease	Comments
Bretagne et al. [30]	32	75	3 (13)	0	7 samples studied in the first 150 days posttransplant
Janitschke et al. [12]	75	71	7 (13)	3 (5)	Serology was found to be useless in the diagnosis of infection and prediction of disease
Martino et al. [31]	106	100	16 (16)	6 (6)	Cord blood transplantation and noncompliance to cotrimoxazole prophylaxis were risk factors for infection and disease
Edvinsson et al. [32]	12 AlloHSCT/21 AutoHSCT	30	2 (17)	1 (8)	
Fricker-Hidalgo et al. [33]	70 AlloHSCT (none received prophylaxis)	57	9 (13) (PCR+ and IgM−)	4 (5.7)	1 seronegative patient developed disease In the 4 patients with disease, 2 had negative PCR but positive IgM serology, and 1 had positive serology before PCR Confirms that serology can be helpful in the appropriate setting
Daval et al. [43]	40 AlloHSCT	100	1 (4)	0	0/25 in pts on cotrimoxazole vs. 1/15 (7%) in pts not on prophylaxis In this study, french expert parasitologists validated a quantitative PCR with a validated competitive internal control
Meers et al. [44]	208 AlloHSCT (none received prophylaxis)	100	12 (6)	6 (3)	Risk factors for infection were myeloablative conditioning, especially with irradiation, and having a seronegative donor Risk factor for disease was having a high parasitic load in PB
Caner et al. [45]	12 AutoHSCT 18 AlloHSCT	100	3 AutoHSCT (25) 4 AlloHSCT (22)	4 (10)	By two PCR methods, this study analyzed a sample of buffy coat from the infused stem cells, as well as posttransplantation monitoring The 4 patients who developed disease had a positive PCR in the donor "buffy coat" samples analyzed; will require confirmation in further studies

usefulness of this approach, provided a sensitive and specific quantitative PCR technique is readily available.

Since histologically proven toxoplasmosis is a very difficult-to-obtain diagnosis, various levels of diagnostic certainty have been proposed, which will aid in the interpretation of further studies in this area [7]. Histologically defined cases are considered as definite cases of toxoplasma disease, PCR-defined cases as probable, and CNS imaging-defined cases as possible cases. Table 42-3 summarizes the modified proposed EBMT definitions.

In HSCT recipients, initial therapy for toxoplasmosis should be administered for at least 3 weeks and the total

TABLE 42-3. EBMT-IDWP definitions for toxoplasmosis after hematopoietic stem cell transplantation (modified from [7])

Toxoplasmosis disease definite toxoplasmosis	Histologic or cytologic demonstration of tachyzoites in tissue samples obtained either by biopsy, bronchoalveolar lavage (BAL), or at autopsy. Isolation of the parasite by culture in these samples would be evidence of disease
Probable toxoplasmosis (PCR-documented) (rarely, positive IgM serology)	Clinical and radiologic evidence suggestive of organ involvement plus at least one positive PCR test from blood, CSF, and/or BAL, but no histologic confirmation and absence of another pathogen, which may explain the findings In patients capable of mounting a humoral immune response, positivity of *T. gondii* IgM (irrespective of IgG results) with negative PCR in a compatible clinical scenario also represents probable disease [33]
Possible toxoplasmosis (imaging-documented)	CT or MRI highly suggestive of CNS toxoplasmosis (as considered by each hospital's neuroradiologists) and response to antitoxoplasma therapy, but no laboratory evidence of toxoplasmosis and absence of another pathogen which may explain the findings
Toxoplasmosis infection	Positive PCR in blood in a patient without any evidence of organ involvement or seroconversion (positive IgM serology) for *Toxoplasma gondii* after transplant in a previously seronegative patient (with or without fever)

Abbreviations: *CT* computerized tomography scan, *MRI* magnetic resonance imaging, *CNS* central nervous system, *PCR* polymerase chain reaction for *Toxoplasma gondii.*

TABLE 42-4. Suggested treatment and prophylaxis for toxoplasmosis in HSCT recipients

Treatment	Dose
Pyrimethamine (*plus* folinic acid)	Oral, 200 mg loading dose, then 50–75 mg q.d. (folinic acid, oral, or IV 10 to mg q.d.)
	Plus one of the following
Sulfadiazine	Oral, 1–1.5 g q 6-8 h
	Or
Clindamycin	Oral or IV, 600 mg q6h

Prophylaxis	Dose
Trimethoprim *plus* sulfamethoxazole[a]	2 double-strength tablets (160/800 mg) per day, 3 days per week or 1 double-strength tablet (160/800 mg) per day, 4–5 days per week or 1 standard-dose tablet (80/400 mg) daily
	Or
Pyrimethamine and sulfadoxine (Fansidar)[a]	2–3 tables per week
Dapsone[a]	100 mg daily
Atovaquone[a]	1500 mg daily
If the above cannot be given, there is in vitro and anecdotal clinical evidence for the following alternatives [34]	
Spiramycin	Daily 25–50 mg/kg/day, maximum 2–3 g/day
Azithromycin	250–500 mg 3 days per week

[a]Also effective for *Pneumocystis jirovecci* pneumonia prophylaxis, and possibly listeriosis, nocardiosis and, in some geographic areas, partly effective in preventing gram positive cocci and gram negative bacillary (enterobacterial and non-glucose fermenting) infections [47]. The dose can be reduced in patients with mild renal insufficiency.

therapy duration should be continued until 4–6 weeks after all clinical evidences of toxoplasmosis resolves. The dosage of the medications utilized may need to be reduced or the regimen changed if side effects occur (primarily rash, diarrhea, or drug interactions). Extended therapy is with pyrimethamine and sulfadiazine or pyrimethamine and clindamycin (Table 42-4). Most patients respond to one or another of these regimens and neurologic improvement of toxoplasma brain involvement usually occurs within 7 days. Because pyrimethamine is a folic acid antagonist the most common side effect is dose-related bone marrow suppression, and patients receiving pyrimethamine should be placed on daily oral dose of 10–15 mg of folinic acid (not folic acid), and have a complete blood count performed twice weekly. Other side effects of sulfonamides include fever, rash, and hepatitis.

Data from AIDS patients suggest that prophylactic cotrimoxazole is useful in minimizing the risk of reactivation of toxoplasmosis, although there are well-reported cases of toxoplasmosis breaking through cotrimoxazole prophylaxis in marrow transplant recipients [4, 5]. Suboptimal dosing may have contributed to some of these "breakthrough"

infections. In a recent review of 47 patients with breakthrough toxoplasmosis, 37 of whom were on cotrimoxazole prophylaxis, indirect observations suggested a significantly lower efficacy of prophylaxis in regimens that use the drug less than three times a week. Thus, using either 1 standard-dose tablet (80/400 mg) daily or double-strength tablets (160/800 mg) 3 or more days per week is the recommended dosing [47], as shown in Table 42-4.

One study in marrow transplant recipients of pyrimethamine and sulfadoxine (Fansidar) described no proven cases of toxoplasmosis in 69 patients receiving this regimen; additionally, no cases of *Pneumocystis jirovecci* pneumonia were reported [16]. Other less well-studied alternatives include dapsone, atovaquone, and azithromycin. Table 42-4 describes the recommended prophylaxis in seropositive patients.

The prognosis of this infection has been considered to be very poor based on the limited published data, with nearly 90% of patients dying from toxoplasmosis (see Table 42-1). This contrasts with the 70–80% response rates observed in patients with AIDS. However, the results from the New York and the EBMT studies suggest that, if appropriately treated, up to 60% of patients may show clinical–radiologic improvement or even a complete response. This highlights the importance for a high index of suspicion for toxoplasmosis in immunocompromised patients for the appropriate diagnostic tests and for starting therapy as soon as possible. Of utmost importance is knowing the patients' serology pretransplant, since the risk of toxoplasmosis in seronegative recipients appears to be very low. However, seronegative patients may also develop toxoplasmosis, either through infection from the donor or primary infection after transplant [12].

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43
Toxoplasmosis After Solid Organ Transplantation

Jose G. Montoya and Carlos A. Gomez

43.1 Introduction

The intracellular parasite *Toxoplasma gondii* infects over one billion humans worldwide and causes disease that can result in significant morbidity and mortality [1]. The term "toxoplasmosis" should be reserved for the symptomatic patient who is ill due to a recently acquired infection (primary infection) or reactivation of a previously acquired infection (chronic or latent infection). Some investigators also refer "toxoplasmosis" as toxoplasma disease [2]. In contrast, the term "*Toxoplasma* infection" is best used for the chronically infected, asymptomatic patient. Over the past 15 years, the epidemiology and our understanding of the parasite have changed considerably. Most notably, untreated water and raw shellfish are now recognized as potential vehicles of *T. gondii* transmission [3, 4] and certain strains of the parasite appear to be associated with more severe forms of illness [5]. Early in the transplantation era, toxoplasmosis was recognized as a life-threading opportunistic infection in SOT recipients [6, 7]. Recently, new studies have contributed to a better understanding of risk factors for toxoplasmosis in SOT especially in non-cardiac recipients [8, 9].

As a result of their significant immune compromise, patients with organ transplants are at a greater risk of developing the most severe forms of toxoplasmosis and, if untreated or diagnosed late, they may die as a result of the disease. SOT-recipients may develop toxoplasmosis by primary infection (primarily acquired orally or via the transplanted organ) or reactivation of *T. gondii* acquired prior to transplantation. In seronegative SOT-recipients, transplant programs should provide patients with educational material regarding preventive measures to decrease the risk of primary toxoplasma infection.

The presence of traditional epidemiologic risk factors for acute infection (e.g., ingestion of undercooked meat, ownership of cats) or even history of an illness suggestive of toxoplasmosis are not sensitive tools to determine whether a patient should be tested for *Toxoplasma* infection or toxoplasmosis [10]. More than 50% of individuals infected with *T. gondii* do not recall experiencing a syndrome consistent with toxoplasmosis, and a similar percentage have never been exposed to the risk factors traditionally associated with acute infection. Thus, to establish whether toxoplasmosis should be included in the differential diagnosis of SOT-patients, *T. gondii* serologic screening should be performed in each transplant candidate and organ donor, in the pre-transplant period, regardless of risk assessed by a careful medical history.

43.2 Etiologic Agent

Three primary forms of the parasite can be easily identified in nature: the tachyzoite, the tissue cyst (containing bradyzoites), and the oocyst (containing sporozoites) (Figure 43-1). The tachyzoite is oval to crescent-shaped, measures 2–3 μm wide and 5–7 μm long, and is responsible for the clinical manifestations observed in patients with toxoplasma disease. The tissue cyst measures up to 100 μm in diameter and is responsible for chronic infection. It is transmitted to humans via consumption of undercooked meat or transplantation of an organ. In humans, this dormant phase can be found in different organs (e.g., heart, liver, kidney, skeletal muscle, eye, and brain) and does not cause symptoms to occur unless it is reactivated by a significant impairment in T-cell and/or B-cell-mediated immunity. To produce symptoms, the bradyzoites contained inside the tissue cyst must undergo a series of metabolic and structural changes, driven by the failing immune system, to transform into tachyzoites. In immunocompetent individuals, tissue cysts can rupture spontaneously and release bradyzoites that are rapidly cleared by specific anti-*Toxoplasma* immunity and therefore almost never lead to clinical symptoms [11, 12]. In organs from donors with chronic *T. gondii* infection, tissue cysts can be transmitted within the transplanted organs. In seronegative SOT-recipients, tissue cysts embedded in the transplanted organ from seropositive donors have the potential for rupture leading to uncontrolled infection. The latter is aggravated in the context of high-degree immunosuppression that follows organ transplantation.

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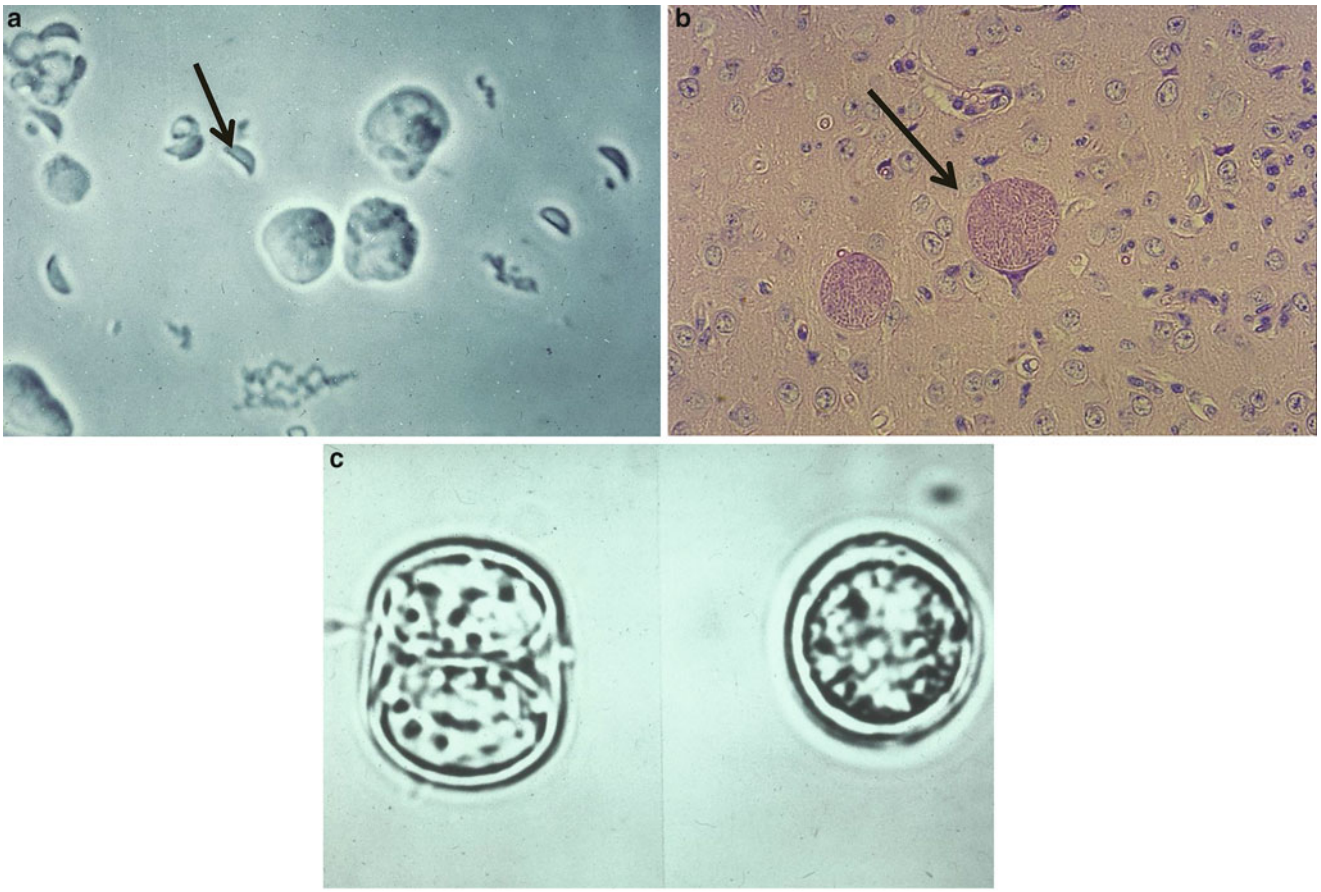

FIGURE 43-1. Main forms of *T. gondii* as found in nature (*arrows*). Tachyzoites (**a**), tissue cysts (**b**) containing bradyzoites and oocysts (**c**) in their sporulated and nonsporulated forms.

Oocysts are shed by all members of the feline family, measure 10–12 µm in diameter in the unsporulated form, and are responsible for the transmission via infected feline feces, vegetables, water, gardening, and contaminated soil. An infected feline may shed as many as ten million oocysts in a single day. Sporulation is required for oocysts to become infectious and occurs outside the cat within 1–5 days depending on temperature and oxygen availability. Sporulation is more rapid at warm temperatures (2–3 days at 24 °C compared to 14–21 days at 11 °C). Oocysts may remain viable for as long as 18 months in moist soil, resulting in an environmental reservoir from which incidental hosts may be infected (Figure 43-1).

Although one major determinant for the severity of toxoplasmosis is the integrity of the cell-mediated immune system, genetic differences among strains may explain the wide range of clinical manifestations observed between different locales [13–21]. In South America, for example, a more aggressive and lethal form of toxoplasmosis has been associated with unique strains (type I, III, and atypical strains). These virulent strains are rarely found in Europe (where type II strains are predominantly found) [5, 16, 22–25]. Disseminated disease, pneumonia, and even death have been observed in immunocompetent individuals infected with this *T. gondii* strain in South America. These emerging and more virulent strains have potentially serious implications for transplant patients who travel to or receive organs from individuals from those areas.

43.3 Life Cycle

Domestic and feral cats, small and large, are the definitive hosts of *T. gondii*. They can be infected with any of the primary forms of the parasite (tachyzoites, tissue cysts, or oocysts). Once in their small intestine, the parasite undergoes asexual or sexual (gametogony) reproduction. Oocysts are subsequently excreted via the feces in the order of millions per day. Humans and other animals (e.g., mammals, chickens, etc.) are incidental hosts and can be infected by ingesting meat (containing tissue cysts) or other food, soil, or water (containing oocysts). An epidemiologic study in the USA revealed that an elevated risk for recent *T. gondii* infection was associated with the following factors: eating raw ground beef; eating rare lamb; eating locally produced cured, dried, or smoked meat; eating raw oysters, clams, or mussels; working with meat; drinking unpasteurized goat milk; and having three or more kittens [4]. Oysters, clams, and

mussels have been shown to carry *T. gondii* under experimental conditions and act as filter feeders that concentrate on the parasite [26, 27]. Moreover, *T. gondii* oocysts remain viable in various species of shellfish under natural conditions [28, 29]. The US study was unable to explain the risk in 48% of the infections and was consistent with data from another multicenter study performed in Europe [30]. Patients may be unaware of their exposures or may have difficulty recalling specific risk behaviors.

Drinking untreated or unfiltered water has emerged as an important risk factor for *T. gondii* acquisition in humans and other animals (e.g., sea otters) in several countries including Canada, Brazil, Turkey, and Colombia [31–33]. In the aforementioned US study, there was a trend toward drinking untreated water being a risk factor associated with acute *Toxoplasma* infection. Some people in the USA are probably infected by ingesting water contaminated with oocysts.

43.4 Epidemiology

The seroprevalence for *T. gondii* infection increases with age and varies considerably by locale and socioeconomic strata [34]. The overall age-adjusted seroprevalence in the USA is around 10% but can be as high as 30% in the Northeast region and higher in certain ethnic groups [35]. Similarly, in the HIV population, the seroprevalence of latent toxoplasma infection ranges from 10% to 45% in the USA. [36]. In the recent decade, a decline in the U.S.-prevalence of *T. gondii* infection has been reported among US born persons amongst 12–49 years of age. In this group, the age-adjusted seroprevalence decreased from 14.1% in 1988–1994, to 9.0% in 1999–2004, to 6.7% in 2009–2010 [37, 38]. Nonetheless, *T. gondii* seroprevalence has substantial geographical variation with higher prevalence (>50%) in Western Europe and South American countries [34, 39–41]. As a more significant participation in the donor-pool is expected in the next decades for foreign-born organs donors, these *T. gondii* seroprevalence disparities may become relevant for transplant programs.

Solid organs from infected donors (D+) can carry and transmit the parasite to their recipients. *Toxoplasma* seronegative recipients (R−) are at greater risk of developing toxoplasmosis when they receive these organs but not receive prophylaxis against the parasite. As the heart is a recognized site of encystation for *T. gondii*, the risk of toxoplasmosis following SOT is higher in heart transplant—especially in mismatched (D+/R−) compared with other SOT-recipients. In a historical cohort, at Stanford University Medical Center, 25% of D+R− patients developed or died from toxoplasmosis because none of them received prophylaxis. The risk of toxoplasmosis in a given transplant program will vary considerably according to the seropositivity of their recipients and donors as well as the degree of exposure that seronegative patients have to *T. gondii*.

We advocate for global screening for *Toxoplasma* antibodies in patients considered for SOT, although this practice varies across different countries due to differences on the incidence rate of toxoplasmosis. For instance, serologic screening for toxoplasmosis is mandatory in France and 11 European countries whereas this practice varies across transplants centers in the USA [42]. Adequate planning for effective chemoprophylaxis against toxoplasmosis relies in the recognition, at the time of transplantation, of cases of D+R− mismatches, particularly for heart transplant recipients. Indeed, rapid availability of *toxoplasma* pre-transplant serologies is desirable in cases where the transplant clinician face severely ill SOT-recipients in whom toxoplasmosis is being considered in the differential diagnosis. Hence, for solid organ transplants, the D+R− high-risk patients should be identified prior to transplantation. Pre-transplant seropositive SOT-patients (R+) are also at risk for toxoplasmosis (by reactivation) following transplantation.

Serologic testing after transplantation to establish risk of disease or to determine if the patient has developed toxoplasmosis is potentially misleading; as post-transplant patients may see their serologies rise or change without necessarily indicating that they have toxoplasmosis disease. In addition, their serologies may not change, become negative, or remain negative in the setting of toxoplasmosis [43] (Table 43-1).

43.5 Heart Transplantation

In a recent multicenter, matched-case control study conducted between 2000 and 2009, the overall incidence of toxoplasmosis following SOT was 0.14%. Heart transplant recipients had a higher incidence of post-transplant toxoplasmosis (0.61%) compared with lower rates in kidney (0.08%) and liver transplant recipients (0.08%) [8]. In this study, a negative *T. gondii*-serostatus prior to transplantation was the only independent risk factor associated with toxoplasmosis. However, the incidence of toxoplasmosis in the group of seronegative heart transplant recipients can range from 25% to 75% in the absence of effective toxoplasma prophylaxis [44–46]. For instance, at Stanford University Medical Center results of serologic testing for toxoplasma were available prior to transplant for 575 D/R pairs; of these, 454 (79%) were D−R−, 84 (14.6%) D−R+, 32 (5.6%) D+R−, and 5 (0.8%) D+R+. Of the 32 D+R− heart transplant recipients, 16 received TMP-SMX and/or pyrimethamine and none developed toxoplasmosis. In contrast, 4 (25%) of the 16 D+R− patients who were not taking either TMP-SMX or pyrimethamine developed toxoplasmosis, and all died of the infection. Of note, none of the 98 who were seropositive patients for *T. gondii* prior to transplantation developed clinical evidence of toxoplasma reactivation [46]. In a landmark study performed at Papworth Hospital in England, Wreghitt et al. implemented a protocol of prophylactic pyrimethamine for all heart transplant recipients with *T. gondii* mismatches

(D+R−). Before the study, out of the first 65 heart transplant cases in the program, 7 were *T. gondii* mismatches (D+/R−), 4 (57%) developed toxoplasmosis, and 2 patients died. After the institution of the protocol, 5 of 37 (14%) patients whom were given prophylactic pyrimethamine developed toxoplasmosis disease; only one was symptomatic, and none died [47]. Later, many studies corroborated the efficacy of trimethoprim/sulfamethoxazole (TMP-SMX) regimens used for prevention of *Pneumocystis* pneumonia (PCP), in the prevention of toxoplasmosis in mismatched heart transplant recipients [45, 48–51]. However, the optimal length and dosing regimen of TMP-SMX prophylaxis for prevention of toxoplasmosis in high-risk heart transplant recipients (D+R−) remain unclear (Table 43-1). Toxoplasmosis in the heart transplant recipient usually occurs in the first 3 months following transplantation and consists of fever, pneumonitis, encephalitis, and myocarditis. Overwhelming *T. gondii* myocardial reactivation can simulate organ rejection and has been diagnosed through endomyocardial biopsy [52].

43.6 Kidney Transplantation

Awareness about the risk of toxoplasmosis disease in renal transplant recipients was raised since the first reported case in 1966 [53], to which was followed by sporadic cases within the early era of transplantation [54, 55]. Nonetheless, toxoplasmosis following renal transplantation remains uncommon, with incidence of 0.08% in one recent multicenter case-control study from Spain [8]. Renoult et al. reported six cases of toxoplasmosis in 373 consecutive kidney transplant recipients in France, from 1989 to 1995 (incidence of 1.6%) [56]. In the first 390 consecutive kidney transplant cases performed at that program, 68.1% were seropositive for *T. gondii* prior to transplantation (R+). Only two of 124 *toxoplasma*-seronegative recipients acquired toxoplasmosis in the post-transplant period; in both cases, the infection was disseminated. An additional review of 31 cases reported in the literature, revealed that the vast majority of cases developed signs of toxoplasmosis within the first 3 months post-transplantation, with only two cases occurring >1 year after transplantation. The most common clinical presentation was fever, pneumonitis, and generalized neurologic signs (headache, confusion, lethargy, and coma). Unfortunately, in 15 cases the diagnosis was performed post-mortem and the mortality rate was 64.5%. Acquisition of the infection through a *T. gondii*-infected-organ was the most common source of transmission [56]. Indeed, toxoplasmosis in kidney transplant recipients occurs mainly by allograft transmission from a seropositive donor, although reactivation of latent infection or primary *T. gondii* infection from environmental sources is also possible [57, 58]. In a series of 34 cases of kidney transplant recipients with toxoplasmosis, the allograft was the source of infection in 12 patients (35%) [9]. Moreover, cases of fatal toxoplasmosis in seronegative renal recipients transmitted by allografts derived from a single-donor with IgG/IgM profile indicative of recent *T. gondii* infection have been reported [56, 59]. Although rare, toxoplasmosis disease can occur upon discontinuation of TMP-SMX (used for *Pneumocystis jirovecii* prophylaxis*)* 6 months following SOT [60] (Table 43-1). Septic shock and chorioretinitis have been reported as the presenting manifestations of toxoplasmosis in kidney transplant recipients [61–63].

Table 43-1. Toxoplasmosis in the setting of solid organ transplantation (SOT)

Type of organ	Most common sources of toxoplasma infection[a]	Risk of toxoplasmosis	Recommended prophylaxis	Recommended duration of primary *Toxoplasma* prophylaxis
Heart/heart–lung	Primary infection via the transplanted organ (D+R−)	Higher in mismatched D+R−	TMP-SMX *or* Pyrimethamine *or* Atovaquone	1 year[b]
Kidney, liver, and multivisceral[c]	– Reactivation of latent infection in seropositive recipients (D−R+ *or* D+R+) – Primary infection with *T. gondii* from environmental sources	– Higher in R(+) with high degree of immunosuppression – Lower in mismatched D+R−	TMP-SMX *or* Atovaquone	1 year[b]

Adapted from [9, 42, 103].

TMP-SMX: trimethoprim/sulfamethoxazole.

[a]The sources of toxoplasmosis in SOT patients are: (1) Reactivation from latent toxoplasmosis infection in a previously infected recipient (R+). (2) Primary infection via the allograft in a previously uninfected recipient (D+R−). (3) Primary infection in a previously uninfected recipient (R−) acquired in the post-transplant period.

[b]Consider prolonging prophylaxis in SOT-recipients who receive antilymphocyte therapies, have concomitant CMV disease, who develop PCP or have undergone treatment for acute allograft rejection.

[c]Includes pancreas and small-bowel transplantation.

43.7 Liver, Pancreas, and Multivisceral Transplantation

Toxoplasmosis has been extensively reported in liver transplant recipients in the context of both primary infection—mainly transmitted through infected allografts harboring *T. gondii*—and reactivation from latent *toxoplasma* infection [9, 64–78]. Nonetheless, toxoplasmosis remains an uncommon complication following liver transplantation. In a cohort of 4872 liver transplant recipients who were followed by 9 years (2000–2009), only 4 (0.08%) developed toxoplasmosis [8]. Similar to kidney transplant recipients, the most common manifestations of toxoplasmosis in liver recipients are fever, pneumonitis, and multisystem organ failure; although cases of chorioretinitis and fatal sepsis have been reported [9, 63, 73, 74, 77]. Most patients developed toxoplasmosis within the first 30 days post-transplantation. The mortality rate remains disproportionally high (9 out of 15 patients, 60%) in a recent case-series, and it is intrinsically associated with timely diagnosis [9] (Table 43-1). Toxoplasmosis in recipients of pancreatic allografts has been reported alone [79] or in the context of multivisceral transplantation [76, 80]. A case of fatal-disseminated toxoplasmosis in a highly immunosuppressed small-bowel transplant recipient demonstrated high-burden disease in both the allograft (jejunum and ileum) and native GI tract (esophagus, duodenum, and colon) [9].

43.8 Immune Response of the Host

The combination of innate, humoral, and cellular immune responses is responsible for controlling both primary and latent infections as well as preventing reactivation. As *T. gondii* is capable of invading any nucleated cell, it provokes an intense pro-inflammatory response that evokes a fine regulation of multiple immune effectors and their signaling pathways [81]. Macrophages, enterocytes, dendritic cells, natural killer cells, T cells, TH1 cytokines (e.g., IFN-γ and IL-12), tumor necrosis factor-α (TNF-α), costimulatory molecules (e.g., CD28 and CD40 ligand), immunoglobulins, and key component of the innate immune system—reactive oxygen species (ROS) and nitric oxide species (NOS)—are crucial for the effective clearance of tachyzoites from peripheral blood, their conversion to bradyzoites, control of the parasite burden, and subsequent formation of cysts in different tissues [81]. Moreover, *Toxoplasma* exhibits inhibitory properties over the apoptotic signaling in infected cells and interferes with the NF-κβ pathways [82, 83]. Immunity in the immunocompetent host has been found to be indefinite probably due to the occasional rupture of individual tissue cysts that provoke a continuous stimulation of the immune system [84].

The greater the defect in T cell-mediated immunity in a transplant patient, the higher the risk for reactivation of latent *T. gondii* infection (i.e., D+/R− heart transplant recipient with enhanced immunosuppression due to acute rejection is at higher risk than a kidney transplant patient).

43.9 Genetic Susceptibility

Certain human leukocyte antigen (HLA) types have been associated with a greater susceptibility to develop central nervous system toxoplasmosis. The major histocompatibility complex (MHC) class II gene DQ3 (HLA-DQ3) has been significantly associated with the development of toxoplasmic encephalitis (TE) in North American Caucasian AIDS patients [85] and with the development of hydrocephalus in children with congenital toxoplasmosis [86]. The HLA-DQB*0402 and DRB1*08 alleles were associated with a high risk of developing TE in South American Caucasian AIDS patients [86].

These studies addressing host genetic susceptibility have not been performed in transplant patients, but they may explain why only 25% of high-risk (D+R−) heart transplant patients [46], and 38% of HSCT patients with previous exposure to *Toxoplasma* [87], develop toxoplasmosis when they do not receive prophylaxis.

43.10 Clinical Manifestations

Toxoplasmosis can result from symptomatic primary infection or reactivation of a previously acquired latent infection. Ninety percent of primary *T. gondii* infections are asymptomatic. Therefore, transplant donors and recipients may have been infected without their knowledge (and, as previously stated, without conventional risk factors for acute infection).

43.10.1 Acute Toxoplasmosis

Symptomatic primary infection may manifest as a painless, nonsuppurative lymphadenopathy. Fever, malaise, and visual symptoms (with retinal involvement) may also be present alone or in combination. Ocular toxoplasmosis causes retinochoroiditis and can result in blurred vision, eye pain, decreased visual acuity, floaters, scotoma, photophobia, or epiphora. Other less common, but well-documented, syndromes have been associated with acute infection including hepatitis, myositis, and myocarditis. More aggressive disease including pneumonia, brain abscesses, and death has been observed in immunocompetent patients in South America. These geographical peculiarities in clinical manifestations may be relevant for the transplant patient who becomes ill after traveling to those areas.

Acute toxoplasmosis in immunocompromised patients is rare but has been described in heart, liver, and kidney trans-

plants when seropositive donors transmit the parasite to seronegative recipients via the infected organ [8, 9, 42]. In this setting, syndromes such as fever, sepsis, pneumonia, brain abscesses, and chorioretinitis have been reported.

43.10.2 Reactivation of Latent Infection

In most individuals, primary infection is followed by a chronic phase during which the parasite appears to be dormant. Clinically meaningful reactivation is not observed in immunocompetent individuals. However, in patients with significantly impaired cell-mediated immunity, *T. gondii* can reactivate and cause encephalitis, chorioretinitis, fever of unknown origin, pneumonia, myocarditis, hepatosplenomegaly, lymphadenopathy, and rash. Although multiple brain abscesses are commonly described in patients with toxoplasma encephalitis (TE) (Figure 43-2), diffuse encephalitis without space-occupying lesions by MRI has been reported with a very high case fatality rate. Fever with pneumonia can be the sole manifestation of toxoplasmosis in immunocompromised patients including SOT and HSCT. Toxoplasmic pneumonitis can present with cough, dyspnea, hypoxia, and diffuse bilateral or localized infiltrates.

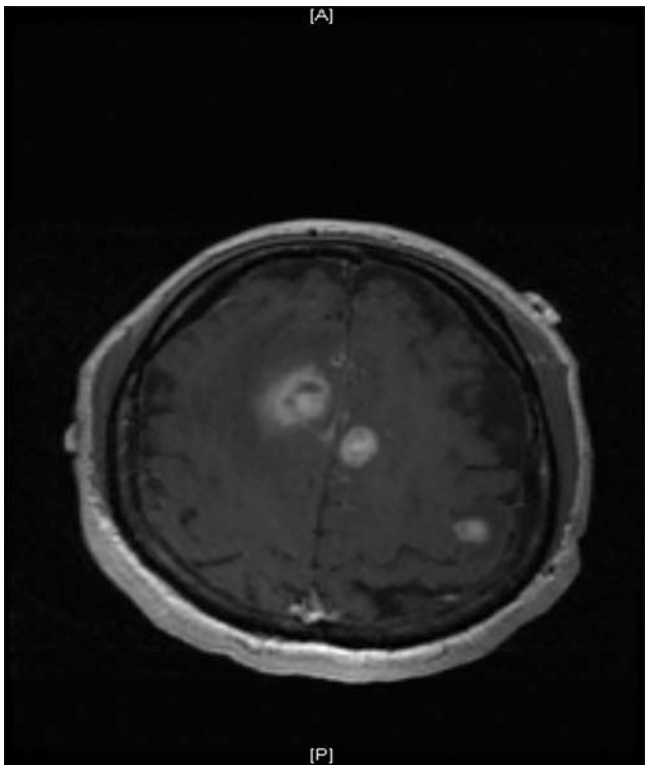

FIGURE 43-2. Contrast-enhanced brain MRI. Fifty-two years old liver transplant recipient (R−) with unknown donor toxoplasma serostatus, showing multiple ring-enhancing lesions suggestive of central nervous system toxoplasmosis. Diagnosis of toxoplasma encephalitis (TE) was established by positive polymerase chain reaction (PCR) in the cerebral spinal fluid (CSF).

In the vast majority of patients, toxoplasmosis in the setting of HSCT is the result of reactivation of an infection acquired in the distant past. A pre-transplant seronegative HSCT patient has a very low risk of toxoplasmosis in the first 100 days of the post-transplant period [2, 87, 88]. Toxoplasmosis in allogeneic HSCT patients is most likely the result of reactivation of a previously acquired infection and occurs more frequently in those who have developed GVHD; in autologous HSCT-patients reactivation occurs rarely (see Chap. 42. Toxoplasmosis after Hematopoietic Stem Cell Transplantation).

Toxoplasmosis in heart transplant patients is most likely to occur in the setting of a D+R− mismatch. Most common clinical presentation of toxoplasmosis in heart transplant recipients includes fever, myocarditis, encephalitis, pneumonitis, and multi-organ dysfunction [8, 42, 44, 46, 89–91]. Toxoplasma retinitis, overwhelming sepsis, and asymptomatic seroconversion in heart transplant recipients have been reported [63, 90, 92]. In a large case-series that include non-cardiac SOT recipients (kidney, liver, pancreas, and multivisceral organ transplants), toxoplasmosis manifested most commonly with fever (77%), respiratory manifestations (29%), neurological dysfunction (26%), bone morrow suppression (26%), and chorioretinitis (10%) [9]. Of note, 85% cases developed disseminated disease, most commonly affecting lungs, brain, bone morrow, and central nervous system [9].

43.11 Laboratory Diagnosis

Available laboratory methods for the diagnosis of toxoplasmosis include direct visualization of the parasite by Wright–Giemsa or immunoperoxidase stains, serologic tests, polymerase chain reaction (PCR), and parasite isolation [93] (Table 43-2). In SOT-recipients with suspicion for disseminated *toxoplasma* disease, detection of *T. gondii* by microscopy and/or PCR, can be attempted in several body compartments such as peripheral blood, bronchoalveolar fluid (BAL), cerebrospinal fluid (CSF), vitreous fluid, ascitic fluid, pleural fluid, bone morrow aspirates, and histologic tissue sections. Of note, conventional stained tissue sections (e.g., hematoxylin and eosin stain) hardly demonstrate tachyzoites. This limitation is easily overcome by the use of immune-peroxidase stain, which uses antisera to *T. gondii*. The immune-peroxidase methods are highly sensitive and

TABLE 43-2. Laboratory diagnosis of toxoplasmosis in transplant patients

Identification of tachyzoites by microscopy
Wright–Giemsa and *T. gondii*-specific immunoperoxidase stains of any body fluid or a "touch" preparation slide of any tissue or biopsy specimen
Identification of tissue cysts in histopathology samples
Hematoxylin and eosin or *T. gondii*-specific immunoperoxidase stains of any tissue or biopsy specimen. Numerous cysts or an associated strong inflammatory response is highly suggestive of toxoplasmosis and not simply *T. gondii* infection
Polymerase chain reaction
T. gondii DNA amplification of the *B1* gene or REP-529 multicopy gene. PCR can be performed in any body fluid including peripheral blood, cerebrospinal fluid, bronchoalveolar lavage fluid, vitreous fluid, aqueous humor, and peritoneal, pleural, or ascitic fluids. PCR can also be performed in tissues, but this method has not been standardized
Serologic tests consistent with a recently acquired T. gondii infection
All transplant candidates and donors should undergo serologic testing for *T. gondii*-specific IgG and IgM antibody tests before transplantation. Positive IgM test results should be sent for confirmatory testing to a reference laboratory specialized in the diagnosis of toxoplasmosis (e.g., Palo Alto Medical Foundation Toxoplasma Serology Laboratory http://www.pamf.org/Serology/). Serologic test results, confirmed by a reference laboratory, that are consistent with a recently acquired infection (e.g., within 3 months of sera sampling) should trigger consideration for treatment of the donor or recipient or postponing the transplant procedure. Serologic tests performed post-transplant may not be accurate
Parasite isolation
Attempts to isolate *T. gondii* can be performed in tissue culture or the peritoneal cavity of animals. Establishing the strain of the parasite may have clinical and prognostic implications
Lymph-node histology
Demonstration of characteristic lymph-node histology can also be used for the diagnosis of toxoplasmosis

specific; and can be applicable to unfixed or formalin-fixed paraffin-embedded tissue sections [94].

The symptoms and/or conventional epidemiologic factors associated with acute *T. gondii* infection are often absent in patients who have been already infected. Thus, if it is clinically important to establish an exposure to *T. gondii*, routine serologic or other tests should be performed regardless of the present illness or epidemiologic history.

43.11.1 Diagnosis of Latent *T. gondii* Infection

Because toxoplasmosis is usually associated with high morbidity and mortality (particularly when diagnosed late), does not have pathognomonic clinical manifestations, and can be treated effectively if diagnosed early, each transplant candidate and donor should be tested before transplantation for *T. gondii*-specific IgG and IgM. Initial serologic testing for *T. gondii*-specific IgG and IgM antibodies can be performed at nonreference or commercial laboratories, but all positive or equivocal IgM test results should be sent to a reference laboratory (e.g., Palo Alto Medical Foundation *Toxoplasma* Serology Laboratory, PAMFTSL; Palo Alto, CA; http://www.pamf.org/serology/;+1-650-853-4828; Toxolab@pamf.org). Transplant candidates and donors that are *Toxoplasma* IgG and IgM negative do not have serologic evidence of prior exposure to *T. gondii* and should be considered to be at extremely low risk for developing toxoplasmosis. Transplant candidates and donors who are *Toxoplasma* IgG positive and IgM negative have been infected for at least 3 months and are at risk for *Toxoplasma* reactivation if they are liver, kidney, pancreas, multivisceral, or HSCT transplant recipients. Reactivation in heart, heart–lung, and lung transplant recipients occurs rarely. Donors who are IgG positive and IgM negative, however, pose a serious risk to their seronegative (*Toxoplasma* IgG and IgM negative) recipients (D+R− mismatch) in the setting of heart, heart–lung, liver, kidney, and kidney–pancreas transplants.

43.11.2 Diagnosis of Acute *T. gondii* Infection and Toxoplasmosis

Serologic test results consistent with a recently acquired infection are diagnostic of acute *T. gondii* infection and highly suggestive of toxoplasmosis in the symptomatic patient but are seldom found in the transplant population. However, an attempt to establish whether the transplant patient has a recently acquired infection should always be attempted by testing the patient's serum for *T. gondii*-specific IgG and IgM antibodies prior to transplantation. If serological testing is performed after transplant, results should be interpreted in light of the results obtained in the pre-transplant period. Acute infection or toxoplasmosis should be suspected in any patient with an equivocal or positive result in any IgM antibody test. A reference laboratory should confirm whether a positive IgM antibody test result is indicative of a recently

acquired infection. Specialized testing at PAMF-TSL includes IgA, IgE, AC/HS, or differential agglutination and IgG avidity assays [93]. Only 40% of positive IgM test results in the USA are confirmed at PAMFTSL to be consistent with a recently acquired infection. If the transplant candidate or donor is confirmed to have a recently acquired infection (e.g., within 3 months of sera sampling), anti-*Toxoplasma* treatment or postponing transplantation (or both) is strongly advised and should be seriously entertained.

The diagnosis of toxoplasmosis from reactivation of a latent infection in transplant patients is rarely established by serologic tests alone. In fact, increasing *T. gondii*-IgG titers and even IgM, can be observed in the absence of clinical manifestations for toxoplasma reactivation. Additional laboratory methods should be used, including stains to visualize the tachyzoite, techniques (e.g., PCR) to amplify *T. gondii* DNA, histological methods to identify characteristic pathological findings or tissue cysts, and parasite isolation.

Visualization of the tachyzoite in any body fluid or tissue is pathognomonic for the diagnosis of toxoplasmosis (in the setting of primary infection or reactivation of a latent infection) and should never be interpreted as a manifestation of latent *T. gondii* infection. In contrast, tissue cysts may simply represent a chronic infection, and in the case of an ill patient, toxoplasmosis may not be the etiology of the patient's syndrome despite the presence of cysts. However, in those cases, the presence of "numerous" cysts or a strong inflammatory response suggests that toxoplasmosis is responsible for the patient's symptoms.

The diagnosis of toxoplasmosis can also be made by PCR of any body fluid (e.g., peripheral blood, cerebrospinal fluid, bronchoalveolar lavage fluid, vitreous fluid, aqueous humor, and peritoneal, pleural, or ascitic fluids). PCR can also be performed in tissues, but this method has not been standardized. The most commonly used gene target is the multicopy B-1 gene. However, the REP-529 multicopy gene has been shown to be more sensitive that the B-1 gene [95]. Quantitative PCR to measure *T. gondii* burden in sequential body compartment samples (peripheral blood, CSF, and BAL) correlates well with favorable clinical response to anti-*toxoplasma* therapy and allows modulation of the immunosuppressive drug regimen [71, 96]. For maximum reliability, clinical samples should be sent to reference laboratories experienced in performing this assay.

Attempts to isolate the parasite from any body fluid or tissue, as clinically indicated, can be attempted at reference laboratories (e.g., PAMF-TSL). If positive, strain typing and genotyping can be used to further study the emerging concept that correlates certain strains with more aggressive disease. Characteristic lymph-node histology can also be used to diagnose toxoplasmosis [97].

43.12 Treatment

Asymptomatic or symptomatic acute *T. gondii* infection or reactivation of a latent infection should be treated immediately in the transplant patient. One hundred percent mortality is observed in SOT patients if toxoplasmosis is left untreated or if treatment is initiated too late. Treatment is also indicated for the transplant candidate and donor with asymptomatic or symptomatic acute infection if transplantation is scheduled to occur within 6 months of the primary infection. In general, higher doses are indicated for the post-transplant immunocompromised patients (Table 43-3). The drug regimen should always include at least two drugs. Pyrimethamine is probably the most active drug against *T. gondii* and is best when used in combination with sulfadiazine. However, studies of TE in AIDS patients reveal that trimethoprim/sulfamethoxazole (TMP/SMX) is equivalent to pyrimethamine/sulfadiazine [98, 99], and according to experienced clinicians, these findings, thus far, translate to the transplant population. Primary therapy should be extended for 6 weeks with longer treatment duration for patients with equivocal clinical or radiographic responses. Following primary toxoplasma therapy, secondary prophylaxis or chronic maintenance (at half-treatment doses) is recommended for SOT-recipients. Duration of secondary prophylaxis must be individualized according to the net-state of immunosuppression and tailored to the perceived risk of toxoplasma reactivation but should be given at least for 1 year.

43.13 Prevention

43.13.1 Primary Prophylaxis for D+/R− SOT-Patients

The (D+R−) SOT-recipients are ideal candidates for anti-*Toxoplasma* primary prophylaxis. TMP/SMX and atovaquone, primarily used by transplant physicians to prevent *P. jirovecii* pneumonia (PCP), have been successful in preventing toxoplasmosis. Effective regimens include one single-strength TMP/SMX tablet daily, one double-strength TMP/SMX tablet thrice a week, or 1500 mg atovaquone daily, with duration of therapy ranging from 3 months to lifelong [45, 48, 49, 51]. Other drug regimens used to prevent PCP (e.g., pentamidine or dapsone alone) are not effective in preventing toxoplasmosis. D+R− SOT-patients have developed toxoplasmosis when TMP/SMX has been discontinued or switched to pentamidine in the post-transplant period and when toxoplasmosis prophylaxis was not addressed [50, 60, 69, 96]. Pyrimethamine (25 mg/day) has also been reported to be effective for D+R− solid organ transplant patients [46]. In high-risk SOT-recipients

TABLE 43-3. Drugs used for the treatment of acute or reactivated toxoplasmosis in transplant donors, candidates, and recipients (primary therapy)[a]

	Transplant donors and candidates with asymptomatic or symptomatic acute infection including clinically active ocular disease, myocarditis, myositis, or hepatitis	Transplant recipients[b] with asymptomatic or symptomatic acute infection or reactivation of latent infection
Preferred regimen		
Pyrimethamine (PO)	50 mg every 12 h for 2 days followed by 25–50 mg daily	200 mg loading dose followed by 50 mg (<60 kg) to 75 mg (>60 kg)/day
Folinic acid[c] (PO)	10–20 mg daily (during and 1 week after therapy with pyrimethamine)	10–20 mg daily (up to 50 mg/day) (during and 1 week after therapy with pyrimethamine)
plus		
Sulfadiazine (PO)	75 mg/kg (first dose) followed by 50 mg/kg every 12 h maximum 4 g/day	1000 (<60 kg) to 1500 mg (>60 kg) every 6 h
or		
Clindamycin (PO or IV)	300 mg every 6 h	600 mg every 6 h (up to 1200 mg every 6 h)
or		
Atovaquone (PO)	1500 mg orally twice daily	1500 mg orally twice daily
Trimethoprim/sulfamethoxazole (PO or IV)	10 mg/kg/day (trimethoprim component) in two to three doses	10 mg/kg/day (trimethoprim component) divided in two to three doses (doses as high as 15–20 mg/kg/day have been used)
Alternative regimens		
Pyrimethamine/folinic acid	Same doses as above	Same doses as above
plus		
Clarithromycin (PO)	500 mg every 12 h	500 mg every 12 h
or		
Dapsone (PO)	100 mg/day	100 mg/day
or		
Azithromycin (PO)	900–1200 mg/day	900 to 1200 mg/day

Preferred regimens: pyrimethamine/sulfadiazine/folinic acid or trimethoprim/sulfamethoxazole.

[a]Assistance is available for the diagnosis and management of patients with toxoplasmosis at the Palo Alto Medical Foundation Toxoplasma Serology Laboratory; +1-650-853-4828; toxolab@pamf.org; http://www.pamf.org/serology/.

[b]After the successful use of a combination regimen during the acute/primary therapy phase, the same agents at half-doses are usually used for maintenance or secondary prophylaxis.

[c]Folic acid should not be used as a substitute for folinic acid (leucovorin).

(D+R−), prophylaxis with TMP-SMX should be extended beyond 6 months in patients that received antilymphocyte therapies, patients with chronic CMV infection, history of PCP or those who have undergone treatment for acute allograft rejection [100–102].

For patients in whom appropriate toxoplasma prophylaxis cannot be given, weekly screening using peripheral blood toxoplasma PCR for 100 days following transplantation, can be considered for D+R− heart transplant patients and R (+) non-cardiac SOT. PCR-positive patients should subsequently be offered effective toxoplasma treatment.

43.13.2 Primary Prevention for the Seronegative Transplant Candidate, Donor or Recipient

Transplant candidates and recipients who do not have serologic evidence of prior exposure to *T. gondii* should be aware of the known risk factors for acute infection. They should adopt basic measures to decrease their risk for exposure to *T. gondii* as much as possible (Table 43-4). Similarly, the same preventive measures apply to seronegative potential donors prior and during the transplant procedure.

TABLE 43-4. Preventive measures to decrease the risk of acute *T. gondii* infection

Reduction of risk of Toxoplasma infection from ingestion of food or water
Cook food to safe temperatures. Do not sample meat until it is cooked. Red meat should be cooked to 160 °F. Whole poultry should be cooked to 180 °F (measured in the thigh)
Peel or wash fruits and vegetables thoroughly before eating
Wash cutting boards, dishes, counters, utensils, and hands with hot soapy water after contact with raw meat, poultry, seafood, or unwashed fruits or vegetables
Freeze meat for several days before cooking to greatly reduce chance of infection
Avoid ingestion of dried, smoked, or cured meat
Avoid eating raw oysters, clams, and mussels
Refrain from skinning animals
Reduction of risk of Toxoplasma infection from the environment
Avoid drinking untreated drinking water
Wear gloves when gardening and during any contact with soil or sand because it might be contaminated with cat feces that contain *T. gondii*
Wash hands thoroughly after gardening or contact with soil or sand
Keep outdoor sandboxes covered
Feed cats only canned or dried commercial food or well-cooked table food, not raw or undercooked meats
Avoid changing cat litter if possible. If no one else can perform the task, wear disposable gloves and wash your hands thoroughly with soap and water afterwards
Litter box should be changed daily if you own a cat. *T. gondii* does not become infectious until 1–2 days after it is shed in the feces
Keep cats indoors
Avoid adopting or handling stray cats, especially kittens

Adapted from Division of Parasitic Diseases (DPD), National Center for Zoonotic, Vector Borne, and Enteric Diseases (ZVED): http://www.cdc.gov/toxoplasmosis/prevent.html.

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44
Parasites After Hematopoietic Stem Cell or Solid Organ Transplantation

Marcelo Victor Radisic and Laura Linares

There are more than 300 parasitic species known to infect humans, but only 5 % have been reported to occur in transplant recipients [1]. Parasitic infections are, in general, neglected diseases, closely related to low socio-economic conditions and poor access to healthcare. World-developing areas suffer most of the burden of parasitic diseases; they also perform a limited number of transplants [2]. This may be one of the reasons to explain scarcity of information on parasitic infection in transplantation, which is mostly based on random case reports, small cohorts, and occasional case-control studies. However, parasitic infections—which may have a deep impact in transplantation outcomes—are increasingly recognized in the industrialized world, and its incidence is expected to grow due to multiple circumstances: Many geographic areas where parasitic infections are prevalent have now active organ transplantation programs; patients from endemic areas with subclinical infections are sometimes referred to transplantation centers in industrialized countries and some patients from developed countries undergo transplantation in highly endemic areas and return home infected; and immigrants from highly prevalent areas are accepted for blood and organ donation without a thorough search for endemic diseases. Also, the use of immunosuppressive regimens without cyclosporine (and without its antiparasitic effect) [3] may partially explain this situation. Lastly, increased levels of leisure travel may enhance risk of exposure when transplant recipients travel to endemic areas.

This chapter addresses the most relevant reported parasitic infections in the transplantation setting.

44.1 Tissue and Blood Protozoa

44.1.1 American Trypanosomiasis (Chagas Disease)

Chagas disease is caused by *Trypanosoma cruzi*, a hemoflagellate protozoan parasite. In 80 % of cases, transmission is vector-borne. However, the infection can also be acquired by contaminated/unscreened blood transfusion (5–20 %), by an infected mother to her fetus (0.5–8 %), by laboratory accidents, sporadically by oral transmission, and by organ transplantation [4, 5].

T. cruzi has a complex life cycle that includes mammals and insect vectors. The vector density and housing standards account for the prevalence of the disease, but immigration from rural to urban communities and also to non-endemic areas has led to an increased non-vector transmission-related risk.

In humans, the disease has an acute and a chronic phase. The acute stage can be asymptomatic or can present only mild clinical symptoms such as a malaise, fever, anorexia, and/or lymphadenopathies, which usually resolve spontaneously in ~8–12 weeks [6]. Acute Chagas disease mortality rate (usually related to acute myocarditis or meningoencephalitis) is 2–6 % [7].

In the acute phase, *T. cruzi* invades different host cells and tissues (e.g., macrophages, cardiomyocytes, fibroblasts, and neurons), producing severe inflammation, but the host's immune response usually leads to parasite control and resolution of this acute phase. However, without specific treatment, the immune response is ineffective to eradicate the infection, and patients become chronically infected with the parasite. The lifelong chronic phase has a long period of clinical latency—the so-called chronic stage without demonstrable pathology [8] (previously called indeterminate phase)—which can last 10–30 years or even lifelong. The infection is evident only by positive serology with intermittent and extremely low levels of parasitemia [9]. About 20–30 % of infected patients in the chronic stage, after several years without symptoms, may progress to symptomatic disease, developing Chagasic cardiomyopathy (90 %), and less frequently, gastrointestinal (15–20 %) [9] and peripheral nervous system disease (~10 %) [10]. The diagnosis of infection in the acute phase relies on the direct identification of the parasites, which are usually present in high titers in blood. Motile *Trypanosomas* can be detected using microscopic examination of the buffy coat, thin or thick blood films stained with Giemsa; or by a concentration method [7] (Strout method [11] or microhematocrit).

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In the chronic phases of the disease, the parasitemia level is low and intermittent, and tissue parasitism is scarce [12]. In this stage, although direct parasite detection can be achieved by blood cultures or xenodiagnosis, these are time-consuming, labor intensive methods, with a sensitivity of less than 50 % [6, 13] making their use impractical and of little or no clinical utility. Low level parasitemia in the chronic phase can be detected by blood polymerase chain reaction (PCR)-based assays, with sensitivity reaching 100 % with repeated sampling [13].

However, the method of choice for Chagas diagnosis in the chronic phase is the detection of circulating antibodies against *T. cruzi* [14]. The most commonly used methods are the enzyme-linked immunosorbent assay (ELISA), indirect hemagglutination (HA), and indirect fluorescent antibody (IFA) test. Sensitivity and specificity are best for ELISA (94–100 % and 96–100 %, respectively), IFA (98 %; 98 %), and particle agglutination (97–100 %; 97–99.5 %) assays, and they are the recommended methods for screening [15]. FDA-licensed screening test for blood or organ donors includes Ortho *T. cruzi* ELISA Test System [16] and ABBOTT PRISM Chagas chemiluminescent immunoassay (ChLIA) with sensitivities and specificities also close to 100 %.

However, in endemic countries, health agencies still recommend that at least two different methods of testing must be positive for a diagnosis of Chagas disease to be reached [7, 17]. Radio-immunoprecipitation (RIPA), Western Blot, immunoblot, and IFA have the highest specificity and sensitivity, and are considered confirmatory tests [7, 15].

During the 1960s and 1970s, two drugs were introduced for treatment: nifurtimox and benznidazole. When these are administered for 30–60 days, both achieve parasitic cure in 60–100 % of acute cases, but cure rates among adults when the disease is in the chronic phase are far lower, in the range from 15 % to 35 % [18].

The adverse side effects of these drugs include dermatitis, peripheral polyneuropathy, weight loss, gastrointestinal disease, hematologic disorders, and an increased incidence of lymphoma [19]. Benznidazole is considered as first-line treatment, because it is better tolerated than nifurtimox; however, some patients tolerate nifurtimox better than benznidazole [20]. Although neither drug is approved in the USA, both can be obtained from the Centers for Disease Control and Prevention (CDC) [21].

Posaconazole and ravuconazole show activity against *T. cruzi*, however, clinical trials investigating treatment of chronic indeterminate Chagas disease revealed their inferiority to the current standard-of-care benznidazole [18, 22].

Allopurinol has trypanocidal properties and may also have a role in Chagas treatment, alone [23] or combined with benznidazole [24].

44.1.1.1 Chagas in Solid Organ Transplantation (SOT)

Chagas endemic area extends from southern USA to northern Argentina in America. However, due to international migration from the endemic areas, Chagas is on its way of becoming a worldwide-health issue. An estimate of ~300,000 infected immigrants are living in the USA, 48,000–87,000 in Spain, and several thousand in other European countries, Australia, and Canada. These infected immigrants may transmit the infection acting as blood or organ donors [25].

Adventure travel is another way to acquire the infection for people from non-endemic areas.

Reactivation of chronic Chagas disease may occur with immunosuppression therapy. In endemic areas, pretransplant serological test for Chagas is routinely performed in transplantation donors and recipients. Awareness of Chagas transmission in non-endemic countries boosted considering or implementing routine serotesting in patients with increased risk, i.e., people from endemic areas, people whose mothers were born in endemic areas, or people who received blood transfusions in an endemic area [26–29].

Different clinical scenarios can be found in the setting of SOT: the patient that needs heart transplantation because of terminal Chagasic cardiomyopathy; the patient with chronic infection that needs a SOT (because of a disease different from Chagas), and the chronically infected donors.

44.1.1.2 The Chagas-Infected SOT Recipient

Since the 1980s, Chagas infected transplantation candidates in the chronic stage without demonstrable pathology have been accepted for kidney transplantation. More recently, liver and kidney–pancreas transplant has also been performed in chagasic recipients.

In most endemic countries, all transplantation candidates are routinely tested for Chagas seroreactivity during the pretransplantation evaluation. This routine should be considered for all candidates with epidemiological risk (recipients who were born, received a blood transfusion or lived for in an endemic area, or whose mother was born in an endemic area). When serotesting is positive, the patients are diagnosed as chronically infected. The following approaches can be considered in this situation: (1) proceed with transplantation; (2) start the patient on specific treatment, with the assumption that either cure or a decrease in the parasite load will be achieved; or (3) search for actual parasitemia with specific detection tests and treat only those with positive results before transplantation.

Failure to detect circulating parasites should be interpreted with caution because low levels of parasitemia and intermittent parasitemia do occur in the chronic stage of the disease;

hence, the direct methods of parasitic detection are usually negative. In addition, there is no documented evidence that pretransplantation treatment leads to better results and the adverse side effects of specific treatment in patients with terminal organ failure make their use almost impossible [30].

A strict protocol to search for reactivation evidence after transplantation is considered the best approach [30–32]. An adequate follow-up modality entails the sequential monitoring for early parasitemia detection with both direct parasitic methods [33] and real-time PCR methods. These new techniques have enabled early reactivation detection, preceding Strout test positivization and clinical signs [32, 34].

Reactivation has been reported in 22–50 % of **kidney transplant** recipients with Chagas disease; it generally occurs soon after transplantation (i.e., in the first 3 postoperative months) [33, 35] but it has also been found as late as 36 months, following immunosuppression intensification [36].

Most reactivated cases have parasitemia with positive Strout tests, but with no clinical manifestations of disease or increase in antibody levels. Severe disseminated disease is highly infrequent, but anecdotal cases of meningoencephalitis [37], tumor-like brain lesions [38], and acute myocarditis [39] have been described. The development of a febrile illness with painful solitary or multiple subcutaneous nodules has been the most frequently described clinical feature of the reactivated illness [33, 35, 36]. Lesions can take different forms—erythema nodosum–like, panniculitis, and ulcers (see Figure 44-1) and are predominantly located in the upper and lower extremities [40]. On histopathologic examination, nodules show nests of intracytoplasmic amastigote parasites that confirm the etiology of the disease. Subcutaneous nodules are not a frequent manifestation of Chagas disease in immunocompetent hosts, and their relatively high incidence in SOT patients could be interpreted as an indication that the reactivation is pathogenically dependent on the tissue pseudocyst reservoir activity [41]. Reports on Chagas disease in kidney transplant recipients lack extensive discussions on the relationship between reactivation and the immunosuppressive regimens, but reported patients received cyclosporine or tacrolimus-based triple therapy; polyclonal or anti-CD3 monoclonal antibodies have been used as induction treatment in many of these cases. The relationship with rejection treatment has not been analyzed in the published reports. Risk of reactivation may correlate with the net state of immunosuppression; therefore, keeping the immunosuppression to a minimum in SOT patients with Chagas disease would be beneficial. Benznidazole (10 mg/kg/day) or nifurtimox (15–20 mg/kg/day) has been administered for 30–60 days. Remission of skin lesions and the disappearance of parasitemia are usually obtained in <2 weeks, without further reactivations documented on long-term follow-up [33, 36].

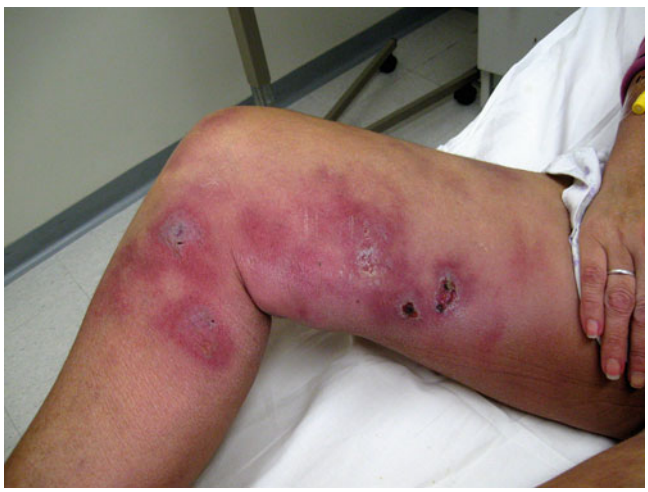

FIGURE 44-1. Skin lesions caused by *T. cruzi* in a kidney transplanted patient.

The experience with **liver transplantation** in chronic Chagas disease is scant. Reactivation has been reported in fewer than 20 % of cases, and it seems to follow the same pattern as in kidney transplantations—parasitemia or subcutaneous nodules with a good response to treatment [42–44].

If liver transplantation is performed in patients with Chagas disease, careful monitoring for possible reactivation—which includes testing for parasitemia and performing biopsies of any subcutaneous lesion—should be implemented.

44.1.1.3 Chagas and Heart Transplantation

Chagas disease is an important cause of end-stage cardiomyopathy in endemic areas. Patients with chagasic cardiac disease are mostly young men who have a life expectancy of 6–13 months from the onset of heart failure [45]. Although Chagas disease was initially considered a relative contraindication for heart transplantation—because of the potential for reactivation in the setting of immunosuppression—it was later demonstrated that patients with chronic Chagasic cardiomyopathy with heart transplantation had higher survival rates than patients who were transplanted because of other heart conditions [45]. Heart transplantation is now routinely performed for this otherwise fatal condition.

Heart transplantation programs in Latin America—mainly in Brazil and in Argentina—have accepted patients with chronic Chagas disease for heart transplantation for the last 25 years [45, 46]. Cardiac chagasic disease is the underlying disease in 35 % of heart transplantations performed in Brazil [47] and 13 % of those performed in Argentina [31]. In the USA, a non-endemic country, seropositive rates in patients with non-ischemic dilated cardiomyopathy who were born or lived for more than a year in endemic area reach up to 19 % in certain areas [48, 49], and so far, 17 patients have been transplanted for Chagasic cardiac disease in the USA [50]. Patients who undergo heart transplantation for Chagasic cardiomyopathy should have a rigorous follow-up protocol, aimed at early detection of parasitemia, which may allow anticipation to clinical reactivation and damage to the heart graft or non-cardiac tissues.

Serial monitoring with microscopy of blood samples and PCR for *T. cruzi* may allow early parasitemia detection, before clinical disease development. PCR-based detection of parasitic DNA has revealed variable levels of sensitivity and specificity, and, among other factors, these variations may be related to parasite's high genetic variability, (which has been the basis to classify it into six Discrete Typing Units (DTUs), TcI to TcVI) [51]. Specificity and sensibility is improved combining 2 o 3 PCR with different genetic targets. A multicenter study for validation of PCR procedures for detection of *T. cruzi* in human blood samples described that the four best performing methods have a sensitivity of 83.3–94.4 % and a specificity of 85–95 % [52].

There is a growing amount of evidence supporting the value of PCR testing for early diagnosis of Chagas reactivation in heart transplanted patients, preceding Strout test positivization and clinical signs. Real-time PCR-based strategies allow measuring parasitic load growth, pre-emptive therapeutic management, and monitoring treatment efficacy [34, 46].

The main clinical features of Chagas reactivation are fever, myocarditis, subcutaneous infiltration with nodules, and rarely disseminated disease [46, 53, 54]. Chagas disease with myocarditis must be differentiated from rejection. On a preliminary observation of endomyocardial biopsy specimens, this may prove to be a difficult challenge [55] because the endomyocardial biopsy specimens show lymphocytic infiltrates with edema and areas of necrosis in both situations. Agent identification provides the diagnosis. Polyclonal antibodies against *T. cruzi* or its antigens (immunohistochemistry) and tissue-based PCRs have been used for diagnostic purposes [56]. PCR sensitivity may differ regarding whether they amplify nuclear (nPCR) or kinetoplast (kPCR) *T. cruzi* DNA. Negative tissue-PCR seems to have a high negative predictive value for Chagas myocarditis, while only nPCR (and not kPCR) may have a positive predictive value [57]. The risk of Chagas reactivation seems to be related to the amount of immunosuppression [53, 58]. Although the use of mycophenolate mofetil (MMF) for maintenance immunosuppression [54, 58], number of rejection episodes and neoplasms have been described as risk factors for reactivation [58], the significance of these findings has been questioned [59]. Patients with reactivated Chagas disease respond very well to benznidazole treatment (10 mg/kg/day for 60 days); nifurtimox could also be effective, but its side effects are considerable. Allopurinol may be considered for treatment because it has good in vitro activity against *T. cruzi* [60]; there is anecdotal experience of its use (dose 600–900 mg/day for 2–3 months) to treat reactivation following heart transplantation, apparently with good results and no side effects [61, 62]. Parasitemia clearance and remission of clinical manifestations are usually obtained in the first treatment week. Some patients may experience relapse—some even several times and many years after transplantation—after the first reactivation episode, with parasitemia or clinical manifestations; however, these individuals have had good responses to the new treatment courses [53, 63]. Mortality related to Chagas disease reactivation has been reported to be 0.3 %, and the survival rates are better than those observed patients who were transplanted because of non-Chagasic cardiac diseases [45].

44.1.1.4 Chagas and Hematopoietic Stem Cell Transplantation (HSCT)

Chagas disease in HSCT may occur due to reactivation of a latent infection [64] (previously acquired by vector-borne transmission, blood transfusion, or congenital transmission); by de novo infection transmitted by blood transfusion during HSCT, or by infection transmitted by the graft. Few patients with Chagas disease have ever undergone HSCT [65–67]. All HSCT candidates at risk for Chagas (Donors or recipients who were born, received a blood transfusion or lived in an endemic area, or donor or recipient whose mother was born in an endemic area) should be serologically screened before they start conditioning chemotherapy. Positive serology is not a contraindication for recipients, but positive donors are not accepted [68] or are accepted only in exceptional circumstances [69]. Patients at risk for Chagas reactivation should be systematically monitored for parasitemia (with PCR—as the most sensitive method—and/or direct methods) and clinical manifestations. Even in non-endemic countries, unknown *T. cruzi* carriers may serve as blood donors. Considering the number of immigrants from endemic countries, a definite possibility does exist that a blood donor may unknowingly be infected. It has been estimated that about 100 million people may be at risk of acquiring the infection this way [7]. Therefore, even outside endemic areas, Chagas disease should be considered in patients with HSCT and unexplained febrile illness [70]. In fact, the first report of Chagas disease in a bone marrow transplant recipient came from Spain; in that instance, the source might have been a blood donor [71]. In patients with chronic Chagas disease, reactivation has been described in 16.6 and 40 % of autologous and allogeneic hematopoietic transplants, respectively (after engraftment and, in sporadic cases, even before the bone marrow infusion during the neutropenia that results from myeloablative preparative regimens). The methodical search for parasitemia allows an early reactivation diagnosis, and prompt, specific treatment can then be initiated with excellent results [67].

44.1.1.5 Donors with Chagas Disease

In countries where the disease is endemic, transplantation teams often have to decide whether to accept a donor with Chagas disease or to postpone the transplantation. Almost ~5 % of all deceased donors in Argentina are chronically infected individuals that are diagnosed by serotesting at the

time of organ procuration [31]. The decision should be made balancing the risk of expected mortality in the waiting list against expected morbidity from eventual Chagas transmission. The likelihood of transmission appears to vary by organ type [72].

Transmission by infected donors to seronegative kidney recipients was reported to be from 0 % [73] to up to 18 % [33, 36]. In liver transplant recipients, infection from positive donors was 0 % (with prophylactic treatment with benznidazole) [74] and from 0 % to up to 20 % (without prophylaxis) [31, 75]. When patients were monitored for early transmission detection, treatment with benznidazole was highly effective, with no mortality attributable to Chagas disease. Based on this information, in Argentina, with appropriate informed consent, organs from infected deceased donors are considered acceptable (with exception of the heart) for non-infected or infected kidney recipients and, eventually, for uninfected lung and liver recipients in emergency situations. Infected living donors should receive trypanocidal treatment for 30 days prior to donation to allow clearance of parasitemia. Donation should take place as soon as possible after treatment completion [31].

Reports of transmission by unscreened deceased organ donors [76, 77] and unscreened blood transfusion [71] in non-endemic countries have also been published. Donor screening should be carefully considered in those areas that have a significant immigrant population from endemic areas.

In summary, patients with Chagas disease are acceptable candidates for SOT and HSCT because the incidence of disease reactivation does not seem to be a deterring factor and because its clinical presentation does not have a life-threatening pattern, furthermore, sequential monitoring may allow early parasitemia detection and pre-emptive treatment implementation. The recipients with Chagas infection must be monitored for parasitemia on a sequential schedule for early detection of reactivation (weekly or every 2 weeks for the first 6 months, monthly thereafter after transplantation, and weekly for 2 months after intensification of immunosuppression) [31]. Infected donors are unacceptable for heart transplantation; however, the use of the other organs is acceptable for infected recipients; for uninfected kidney recipients; and, eventually, for uninfected lung and liver recipients in the emergency list. These patients should not only be monitored for disease transmission, but also be fully informed and must provide written consent.

When transplantation is being performed to a non-infected patient who resides in or who moves to an endemic area, this individual has the possibility of being exposed to vector transmission, and acquire post-transplant Chagas infection. This situation has been reported [78]. Possibly, the best approach would be to search for parasitemia at regular intervals. Seroconversion is also diagnostic, but it may be delayed or may not occur at all in these patients.

44.1.2 Leishmaniasis

Leishmaniasis is caused by a heterogeneous group of protozoan parasites, and comprises a variety of clinical syndromes. This zoonotic infection is transmitted by infected sandflies in tropical and subtropical climates in the Mediterranean basin in Europe, Asia, Africa, and America [79].

Clinical forms of leishmaniasis include cutaneous leishmaniasis (CL); mucocutaneous leishmaniasis (MCL); and visceral leishmaniasis (VL). The most severe form is visceral leishmaniasis (VL), which is typically fatal if untreated.

An estimate of 350 million people are at risk of acquiring the infection [80], and approximately 0.2–0.4 million VL cases and 0.7–1.2 million CL cases occur each year [79].

Infection is usually asymptomatic in immunocompetent host. Viable parasites persist lifelong in the host [81], but derangement of cell-mediated immunity may allow reactivation of the disease, which may appear as late as 30 years after the initial infection [82]. As should be expected, disease incidence is higher among the low socioeconomic, immunosuppressed, or malnourished population.

With increased travel and access to advanced medical care in developing countries, the leishmaniasis burden in immunosuppressed individuals will probably continue to rise. The true incidence of the disease is underestimated, especially in hyperendemic regions [79]. Imported leishmaniasis is being increasingly reported in Europe [83–88].

44.1.2.1 Leishmaniasis in Transplantation

Transplanted patients may acquire leishmania infection as primary infection after transplantation takes place; they may have reactivation of latent infection after transplantation; or may acquire the disease from the graft [82, 89]. Clinical manifestations of leishmaniasis in transplant recipients appear similar to those observed in normal hosts, although some atypical features may be present.

44.1.2.2 Visceral Leishmaniasis

Visceral leishmaniasis (VL) is caused by *L. donovani* complex (*L. donovani, L. infantum*, and *L. chagasi*). Leishmaniasis should always be considered as differential diagnosis in patients with fever who live, have lived in endemic areas, or who have a history of travel to them, even in the remote past. Visceral leishmaniasis presents at a median time of ~30 days, but presentation as early as 7 days [82] and as late as 13 years [90] after transplantation has been observed. Reactivation of previously acquired infection is the most frequent disease mechanism in transplanted patients [91, 92]. Visceral leishmaniasis incidence in endemic areas has been described in 0.1–0.5 % of transplanted patients [93] occurring in all types

of transplantations: Kidney [94–96], kidney–pancreas [97, 98], liver [99–101], heart [99, 102], lung [93], and bone marrow [103–108].

The main clinical manifestations are fever, splenomegaly, and pancytopenia [109]. The presenting symptoms are often atypical because anemia and leukopenia may be absent and splenomegaly may develop late in the course of an unnoticed infection [94]. Fever of unknown origin, diarrhea, and malabsorption caused by infiltration of the gastrointestinal tract [110, 111]; interstitial pneumonitis with fever and pancytopenia [112]; and kidney graft's interstitial nephritis [113, 114] may also occur.

Immunosuppression is one of the most important risk factors for overt clinical disease, and can also alter disease presentation and treatment response [115]. High-dose prednisone in the preceding 6 months [93], cytomegalovirus infection after transplantation, and living with cats [93] have been reported as risk factors for VL.

Post–kala azar cutaneous disease may occur in a subset of patients after treatment of visceral leishmaniasis. This condition is characterized by macular, papular, or nodular lesions in the face, trunk, or limbs; amastigotes are present in macrophages within the lesions. Post–kala azar cutaneous disease with distinctive tongue involvement has been reported in two transplant recipients [99, 116].

44.1.2.3 Cutaneous and Mucocutaneous Leishmaniasis

Cutaneous and mucocutaneous presentations are rare in the transplant setting and a protracted time interval may occur between transplantation and disease manifestations [82, 117].

Diffuse cutaneous leishmaniasis and mucosal leishmaniasis (sometimes with exclusive tongue involvement) have been described [118–122].

44.1.2.4 Diagnosis

Even when leishmaniasis is actively sought using appropriate methods, the diagnosis can be elusive, and the examination of multiple samples may be needed [109].

Definitive diagnosis requires the identification of the parasite in a smear or tissue culture (usually bone marrow or spleen). Aspirated material should be inoculated into Novy-MacNeal-Nicolle or other parasitic growth media and the remainder should be used to prepare a Giemsa-stained smear. Culture sensitivity is 60–85 % [123]. Bone marrow microscopy sensibility in transplanted patients is ~98 % [82]. Serological studies (IFA, ELISA, and direct agglutination test) are usually positive in transplanted patients with leishmaniasis (92 %) [82]. However, positive serology does not distinguish past from present infection, and may have cross-reaction with Chagas disease, malaria, and other infections. The recombinant kinesin antigen (rK39) is a useful antigen in ELISA assays achieving high specificity [124] although sensitivity in transplant recipients is currently unknown. Serological results interpretation demands a context of clinical and epidemiological information.

When available, urinary antigen test and PCR techniques are extremely useful. Quantitative or semiquantitative PCR assays have shown a high diagnostic sensitivity in a limited number of patients, allowing measurement of blood parasitic load; therefore, these tests could be used as surrogate markers of disease activity and response to treatment [82].

Cutaneous and mucosal leishmaniasis is best diagnosed by histopathological examination and culture of a small wedge or punch biopsy specimen taken from the margin of the lesion at a site that is not ulcerated; touch preparations should also be performed because their diagnostic yield is often superior to that from the histopathological examination [80]. After a parasite has been identified in tissue samples or culture, speciation can be performed through isoenzyme analysis or species-specific monoclonal antibodies. Quantitative or semiquantitative PCR assays have high diagnostic sensitivity when applied to histopathological specimens [125].

44.1.2.5 Treatment

Visceral leishmaniasis can be treated with amphotericin B, pentavalent antimonial drugs, paromomycin, and miltefosine (the first oral drug for treatment of VL). Liposomal amphotericin has the highest therapeutic efficacy and the most favorable safety profile and should be used whenever possible [126]. Liposomal amphotericin B has been used as the first-line treatment in a small number of kidney transplant recipients (total dose administered was 20–40 mg/kg) with optimal results and no significant toxicity or relapses [127]. Acceptable second-line therapies include conventional amphotericin B deoxycholate or pentavalent antimonial drugs (with close monitoring for toxicity), miltefosine, and paromomycin.

The treatments have several drawbacks such as duration, administration, high costs, and adverse effects. Drug resistance has also been reported, so whether combination therapy could be an alternative needs to be evaluated [128, 129].

Immunosuppression may be temporarily reduced during the initial phase of treatment in severe cases. Bacterial superinfections are a common complication, and they are the important cause of death. Up to 36 % of patients may present bacterial or cytomegalovirus infections [93], and the mortality rate in transplant recipients is nearly 30 %. Patients should be closely monitored because relapse and recurrence may occur in approximately 30 %. Repeated spleen measurement has been proposed as both a marker of cure and a recurrence predictor [109].

Secondary prophylaxis with intermittent weekly amphotericin [130], daily fluconazol [100], and monthly

meglumine antimoniate [99] has been reported. It has also been suggested that allopurinol could be effective to prevent relapses [131].

For cutaneous or mucocutaneous leishmaniasis, pentavalent antimony compounds should be considered first-line therapy for most patients.

44.1.2.6 Prevention

In endemic areas, serological studies of the donor and recipient may allow transmission/reactivation recognition and close monitoring implementation for signs and symptoms of infection. Primary prophylaxis for VL in asymptomatically infected individuals is currently not recommended [132]. A Brazilian study showed that none of the liver transplant recipients who were found to be *Leishmania*-PCR positive at the time of transplantation or received a PCR-positive organ developed VL over a median follow-up of 24 months, without any prophylaxis being given [133]. Protective measures to prevent sandfly bites are recommended for immunosuppressed individuals living in or traveling to *Leishmania*-endemic regions [83].

44.1.3 Malaria

Malaria is an acute febrile disease caused by *Plasmodium falciparum*, *P. ovale, P. vivax*, *or P. malariae*. Recently, *P. knowlesi* was also recognized as a human pathogen [134]. It is the most prevalent vector-borne disease in the world, and endemic in many tropical regions of Asia, Africa, and Central-South America [135]. Estimates of infections ranging between 130 and 390 million, and 2.6 billion individuals at risk of infection [136]. In Europe, an autochthonous malaria outbreak was reported in Greece in 2009 [137].

44.1.3.1 Malaria in Solid Organ Transplantation

Malaria in transplanted patients—a rare event in spite of the disease's high worldwide prevalence—has been related to: blood transfusions, either during the course of the terminal organ failure or in the immediate pretransplantation or post-transplantation period; transmission by the donated organ; reactivation of an old infection, and eventually, *Anopheles* mosquito inoculation. In developed countries, the disease is seldom seen, but it should be considered when looking after transplant recipients who present with an unexplained febrile picture and have resided in or have visited areas where the disease is endemic or have received an organ from a donor living in or originally from such areas. Careful malaria past history investigation is recommended due to the possibility of persistent disease, that can reach many years for *P. falciparum*, *P. vivax*, and *P. ovale* [138] and as long as 44 years for *P. malariae* [139].

Few cases of post-transplantation malaria have been published. It may be speculated that not all diagnosed cases get reported and that the number of post-transplantation malaria cases might grow because of increasing tourism; refugee and immigration movements; and living, unrelated organ donation in countries where the disease is endemic [140]. Transmission from the graft seems to be the main form of acquiring the disease in solid organ transplant recipients [141], although some cases have been traced to blood or blood products transfused to the recipient, even those occurring well before transplantation [142]. In some other cases, the source of malaria has not been clearly established. All of *Plasmodium* species (*P. knowlesi* excepted) have been implicated as the causes of infection in the transplantation setting [140]. Depending on the infecting species, speculation on the pathogenesis of transmission may arise. *P. malariae* infection may persist in the bloodstream for long periods, enabling transmission from an asymptomatic blood or organ donor. *P. vivax* and *P. ovale* hypnozoites may persist in the liver; the infection might be transmitted by a graft liver and a "relapse" of malaria would be seen in the recipient [143]. In addition, if procuration of organs other than the liver occurred during a subclinical relapse of the infection, infected erythrocytes might remain in the allograft despite perfusion and these may be the cause of disease in the recipient [141]. *P. falciparum*-infected erythrocytes have been demonstrated to remain in the capillaries, and reports have indicated that this cytoadherence is resistant to removal by flushing and irrigation at the time of organ preparation for engraftment, thus providing a possible route of infection transmission [144].

Clinical manifestations of the disease have occurred in the early post-transplantation period and have been described in kidney, liver, and heart recipients [141, 143]. In endemic areas, post-transplantation malaria may occur, but the incidence of the disease in transplant recipients is similar to the incidence in the general population [145].

Fever has been reported as the most frequent presenting symptom, but it did not always have the typical paroxysmal or cyclic pattern [146, 147]. Malaria may cause high creatinine levels—that revert with antimalaria treatment—in kidney transplant recipients [147]; and high transaminase levels in liver recipients, making differentiation from rejection critical [143].

Early diagnosis and the specific standard treatment administration are essential for a good outcome [141, 148]. However, serious disease may be observed in *P. falciparum* infection, and when drug toxicities or other infections complicate the clinical course. Special attention may be needed when quinine is used for treatment because it may interfere with cyclosporine, decreasing its blood levels [149].

44.1.3.2 Malaria in Hematopoietic Stem Cell Transplantation

The few reported cases of malaria after HSCT include both autologous [150] and allogeneic transplants [151–157]. The information is anecdotal, originating from case reports only. Although pretransplant screening for malaria does not avoid

the need for clinical surveillance, it could decrease the incidence of transplant-related malaria. Reported cases include patients with geographic exposure and no history of disease [149, 150]; patients and donors with a history of treated disease [155]; patients with no history of disease but with a large number of blood transfusions performed in endemic areas [152]; patients with remote geographic exposure and donors living in an endemic area with negative smears before procuration [153]; and unrelated bone marrow transplants from volunteer donors with a disease history that was undisclosed at the time of donation [151]. Reactivation of infection in bone marrow transplant recipients has also been reported. The diagnosis can be difficult in this particular setting when the aplasia caused by conditioning therapy has not yet reverted. As the number of circulating red blood cells is still low, parasitemia detection by blood smears is more troublesomet. The disease is much more severe in splenectomized patients because the spleen is responsible for removing parasitized cells from circulation.

44.1.3.3 Donor-Derived Malaria

Donor-derived malaria to multiple SOT recipients from a single donor has been reported [158, 159]. Recognition of transmission to one recipient usually raises the alarm to investigate transmission to the others [160]. Donor-derived malaria also may occur in HSCT [154]. When the risk of transmission from an infected donor is acknowledged, recipients should be studied for transmission with the most sensitive technique for parasitemia detection (PCR, and/or blood smears when PCR is not available). Prophylactic treatment is recommended to avoid transmission, or, if asymptomatic donor-derived infection is documented, pre-emptive treatment should be administered [159, 160].

Living donors diagnosed with malaria should be treated before donation to avoid transmission. Deceased donors with malaria should not be accepted.

44.1.3.4 Diagnosis

Malaria diagnosis in transplanted patients is usually made by identifying the parasite in thin or thick blood smears in patients with fever, unexplained hemolysis, and thrombocytopenia [145]. Quantification parasitemia level, expressed as the percentage of infected erythrocytes, is important as a direct correlation exists between parasitic load and disease severity [161]. *Plasmodium* species identification (based on parasite's morphology) is important for choosing specific treatment.

Parasitemia detection by microscopic observation of thick or thin blood smears has been recommended to rule out malaria infection in donors, but false-negative results do occur in asymptomatic patients [162]. Plasmodia antigen detection based on immunochromatography may be negative when parasitic burden is low. DNA detection by PCR is highly sensitive, detecting as few as one parasite per microliter, and allows species identification [163]. Specific antibodies can be investigated by latex agglutination and enzyme-linked and indirect fluorescent antibody assays. Positive antibody titers >1/64 are indicative of current or past infection [139].

44.1.3.5 Treatment

Plasmodium species identification and knowledge of their geographic distribution and sensitivity patterns are essential for decision making. Treatment should be initiated as soon as possible as the disease may be lethal in a transplanted patient. Treatment of choice for severe *P. falciparum* cases is intravenous artesunate, or if not available, intravenous quinine or quinidine administered in conjunction with clindamycin, doxycycline, or tetracycline [164]. *P. vivax, P. malariae, P. ovale*, and uncomplicated *P. falciparum* infection in chloroquine-susceptible regions should be treated with chloroquine. In areas of chloroquine resistance, therapeutic options include atovaquone–proguanil, a quinine-based regimen, or mefloquine [148].

44.1.3.6 Prevention

Routine screening with PCR, serology, or blood smear examination should be implemented for all patients and donors with epidemiological risk. In the absence of laboratory infrastructure, prophylactic treatment might be indicated to reduce the risk of infection [165].

Patients from non-endemic areas traveling to endemic areas should have pre-travel consultation and appropriate chemoprophylaxis to lessen the risk of acquiring malaria [166].

44.1.4 Babesiosis

Babesiosis is a zoonotic disease caused by *Plasmodium*-like protozoans (*Babesia microti, B. divergens, B. duncani,* and *B. MO-1*). Humans are accidental hosts, and transmission may occasionally occur via a tick vector or by blood transfusions. Babesiosis hallmarks are fever and hemolytic anemia. It is usually a mild disease in healthy people, but it can be fatal in asplenic and immunocompromised patients. *B. microti* infection is endemic in some regions of the USA, while *B. divergens* is endemic in Europe. Diagnosis is made through parasitemia detection by PCR or intraerythrocytic parasite identification in thin blood smears, and through serology by indirect immunofluorescent antibody testing [167]. High azithromycin doses (600–1000 mg) combined with atovaquone (750 mg twice daily) may be used for the treatment of immunocompromised patients [168]. Clindamycin and quinine are also effective. Partial or complete exchange transfusion is recommended in case of high

parasitemia (>10%), severe anemia (hemoglobin <10 g/dL), pulmonary, kidney, or hepatic failure [167]. In immunocompromised patients, therapy should be >6 weeks, including 2 weeks after parasites are no longer detected on blood smear [167]. Cases reported in transplant recipients have been traced back to pretransplant blood transfusion in an HSCT recipient [169]; to post-transplant blood transfusion in a kidney recipient [170], and to tick bite in both an SCT recipient [171] and in a splenectomized kidney transplant recipient, years after transplantation [172].

44.2 Worms

44.2.1 Strongyloidiasis

Strongyloides stercoralis is a human parasite, although dogs, cats, and apes can also be infected [173]. An estimate of 80–100 million persons are infected worldwide [174, 175]. The parasite is endemic to the tropics and subtropics, and it has also been reported worldwide excluding only the far north and south [175]. *S. stercoralis* is a nematode with a complex life cycle. Filariform (infective) larvae penetrate human skin and are carried to the lungs through the bloodstream. They reach the bronchial tree and are carried by respiratory secretions to the pharynx and swallowed, entering the small intestine where they can develop into adult worms. *Strongyloides* has the unique ability to complete the entire cycle within one host, thereby leading to autoinfection. In autoinfection, adult female worms within the intestine produce eggs that hatch into non-infective rhabditiform larvae which may subsequently molt into infective filariform larvae. These can then penetrate intestinal mucosa or perianal skin, migrate to the lungs and begin the cycle again. As a consequence, lifelong-parasite persistence in the host can occur despite the absence of external sources of infection [176].

Asymptomatic patients who acquired the infection decades before may develop life-threatening disease in the setting of immunosuppression or immunosuppressive medication.

According to the immunologic response of the host, the clinical spectrum of strongyloidiasis can vary from asymptomatic or mild disease to an amplification of the normal life cycle of the parasite known as ***Strongyloides* hyperinfection syndrome**. The molting of non-infective (rhabditiform) into infective (filariform) larvae is accelerated under immunosuppression, and a massive number of larvae from the intestinal lumen or the perianal skin autoinfect the host, traveling through the venous system to the lung and back to the small bowel. Clinical manifestations of this process are gastrointestinal (abdominal pain, diarrhea, ileus, obstruction, and gastrointestinal bleeding) and respiratory symptoms (cough, dyspnea, wheezing, and respiratory distress syndrome).

Disseminated strongyloidiasis occurs when larvae spread through the venous system to other organs (such as the urinary tract, liver, brain [177], and skin [178]). Complications of disseminated *Strongyloides* include bacteremia and meningitis, usually due to enteric Gram-negative bacteria which are thought to be carried to the lymphatic or mesenteric circulation by their attachment to the infecting larvae [179] or by passing through the disrupted mucosal barriers [176].

44.2.1.1 Strongyloidiasis in Transplantation

Strongyloidiasis has been described in solid organ [179, 180] and stem cell transplant recipients [181, 182] and has been attributed in most cases to reactivation of an old dormant infection. However, donor-derived strongyloidiasis has also been described in kidney, kidney–pancreas, liver, heart, lung, and intestine recipients. High-dose corticosteroid preconditioning of deceased donors may have a role increasing the risk of transmission [183].

Almost all of reported patients with reactivation have had a history of travel to or residence in endemic areas. Many of them had neither eosinophilia nor gastrointestinal symptoms before transplantation [179, 180]. Immunosuppression can transform a chronic, asymptomatic infection into hyperinfection syndrome, and disseminated strongyloidiasis. This transformation usually occurs in the initial months after transplantation when immunosuppression is most intense.

In the reported cases of strongyloidiasis in kidney [180, 184–187], liver [188, 189], and heart transplant recipients [190–192], it was found that most patients had hyperinfection syndrome or disseminated disease, being intestinal strongyloidiasis far less frequent. Systemic polymicrobial bacterial infections were observed in approximately 60 % of the patients [179].

Only a few cases of strongyloidiasis have been described in autologous and allogeneic stem cell transplantation patients [181, 182, 193], but they have been associated with a poor prognosis, especially when they evolve into a hyperinfection syndrome [193]. The onset of symptoms of *Strongyloides* infection in allogeneic HSCT patients seems to be earlier than in solid organ transplant recipients (median time 21 days) perhaps as a consequence of the more intense immunosuppression regimens [193].

Strongyloides hyperinfection syndrome is infrequent, and diagnosis is often delayed. However, it should be considered in the presence of diarrhea, abdominal pain, and fleeting pulmonary infiltrates with or without polymicrobial bacteremia. Because of the large parasitic load, filariform larvae can be easily observed in stool and respiratory samples (such as sputum, bronchoalveolar lavage fluid, and brushings). In disseminated disease, larvae can be observed in blood, cerebrospinal fluid, pleural fluid, urine, or biopsies of affected tissues. Duodenum biopsies may demonstrate rhabditiform larvae in patients who undergo upper gastrointestinal endoscopy [194].

44.2.1.2 Treatment

Ivermectin is considered the treatment of choice for uncomplicated strongyloidiasis as two single oral 200 mcg/kg doses of ivermectin administered either on 2 consecutive days or 2 weeks apart [195, 196]. Adverse effects, such as diarrhea, pruritus, anorexia, and increased transaminases levels, are infrequent and usually mild. Albendazole (400 mg twice daily for 3 days) has a primary cure rate of only 45–75 % [197] making it a second-line therapy. Thiabendazole is the agent with the most extensive clinical experience, but is also the least satisfactory of all available drugs due to frequent relapses and toxicities [176, 177].

The experience with ivermectin for the treatment of hyperinfection or disseminated disease in transplant recipients is still scarce, and reports describing clinical failure have been published [198]. Daily doses should be administered until larvae eradication from clinical samples with additional doses for 7–14 days to reduce the risk of relapse [194]. Retreatment is indicated if significant symptoms or eosinophilia return [148]. In many cases, combination or sequential ivermectin and albendazole treatment was used [199–203]. Severe strongyloidiasis may preclude oral treatment due to intolerance or malabsorption. In these cases, as in those refractory to oral therapy, use of subcutaneous (off label) veterinary formulation of ivermectin (200 μg/kg, daily, divided doses, each arm, until negative stool exam persists for 2 weeks) [204] should be considered [188, 197, 202]. Despite anecdotal success with rectal ivermectin treatment [205], absorption from the rectum may be insufficient to achieve therapeutic levels [206, 207].

S. stercoralis and HTLV-1 co-infection is a special situation that requires protracted therapy because no treatment reliably cures strongyloidiasis in this setting [208].

44.2.1.3 Pretransplant Screening and Prevention

Recipients and living donors residing or with a history of residence in areas where *S. stercoralis* is endemic need to be screened and treated before transplantation. Deceased donors with epidemiological risk should also be tested and prophylactic treatment (ivermectin, two oral 200 mcg/kg doses 2 weeks apart) [184] should be administered to recipients to avert donor-derived transmission, as strongyloidiasis may be transmitted via the graft [183].

Serological assays, when available, are the method of choice for screening. ELISA methods to detect IgG antibodies directed against antigens of *S. stercoralis* are highly sensitive (97 %) and specific (close to 100 %) [209]. False-positive results may occur, related to cross reactivity with other helminthic infections. The sensitivity and specificity of serologic tests in immunocompromised patients are unknown [176] and false-negative tests have been reported [210, 211]. Positive serologic tests may be positive because of a resolved or unresolved previous *Strongyloides* infection. Thus, ideally, examination of fecal samples should follow a positive serologic test [212]. Direct parasitological detection in stools varies in sensitivity because of low numbers and intermittent release of larvae. A single stool examination may not identify more than 70 % of positive cases [213]. The diagnostic sensitivity increases if serial stool samples are studied [176, 177], and the use of specialized methods can improve the diagnostic yield to close to 100 %. The agar plate culture is the method of choice for strongyloidiasis diagnosis because its sensitivity can be greater than 90 %, but multiple stool samples are needed to confirm negative results [214]. Although it is not widely available, reported sensitivity of real-time PCR in fecal samples is 100 % [215]. Parasites may also be sought for in duodenal fluid aspirated by duodenoscopy or obtained with the string test (enterotest capsules) [216].

Empiric treatment may be considered for transplantation candidates with unexplained eosinophilia and who reside in or have traveled to—even in the remote past—endemic areas and in those in whom strongyloidiasis is suspected on clinical grounds before the initiation of immunosuppressive therapy [148, 179]. Transplant recipients from endemic areas should be advised against walking barefoot, and they should be warned about the possibility of acquiring strongyloidiasis from endemic environments.

44.2.2 Schistosomiasis

Schistosomiasis is a parasitic disease caused by worms of the genus *Schistosoma*, affecting at least 260 million people worldwide [217].

The life cycle of the parasite is complex, requiring intermediate and definitive hosts. The infection is acquired through the skin when in contact with contaminated fresh water. The parasite reaches through venous circulation the liver or the bladder, maturing into adult worms which shed eggs that reach the stools or the urine.

Most human infections are caused by *Schistosoma mansoni* (Africa and South America), *S. japonicum* (East Asia), and *S. haematobium* (Africa and the Middle East).

S. mansoni and *S. japonicum* can lead to intestinal and hepatic complications, while *S. haematobium* predominantly leads to kidney and bladder sequelae. Less common, *S. mekongi* and *S. intercalatum* can lead to intestinal and/or liver disease.

Chronic schistosomiasis is the consequence of the granulomatous immune response to retained parasite eggs, and, in the long-term, it may result in permanent and progressive tissue damage.

End-stage liver or kidney failure may be caused by schistosomiasis. Therefore, patients from endemic areas waiting for liver or kidney transplantation should be thoroughly tested for schistosomiasis and treated before transplantation.

44.2.2.1 Schistosomiasis and Kidney Transplantation

Chronic *S. haematobium* infection may lead to urinary tract fibrosis, with obstruction and eventually end-stage kidney failure, whereas *S. mansoni* infection can induce terminal glomerular disease by the deposition of immune complexes [218]. More than 150 infected patients, some of whom had mixed infections, have undergone kidney transplantation [218, 219]. Patients transplanted for schistosomiasis have long-term outcomes similar to those observed in patients who underwent kidney transplantation for other diseases [219]. Urinary tract complications occur in 15 % (e.g., urinary anastomotic or ureteral obstruction, ureteral necrosis, urinary tract infection) of these patients. This may be related to poor tissue healing related to inflammatory changes in the setting of chronic urinary tract schistosomal infection [219]. Reactivation is infrequent [220, 221]; this has been attributed to specific pretransplantation treatment and to the possible antiparasitic effect exerted by cyclosporine or its non-immunosuppressive metabolites [222]; this potential effect [223, 224] could also explain the low level of re-infection in transplant recipients. Lack of treatment in the pretransplantation period seems to be associated to *Schistosoma* disease recurrence [219], although this association is not universal [225].

44.2.2.2 Schistosomiasis and Liver Transplantation

S. mansoni, S. japonicum, and *S. mekongi* infection may lead to chronic liver disease by way of immune-mediated damage and parasite eggs direct liver toxicity, causing pipe-stem fibrosis (pipe-shaped fibrosis around the hepatic portal veins, associated with large numbers of schistosome eggs) [226].

Liver transplantation has been performed mainly in patients co-infected with hepatitis C virus (HCV). Whether co-infection with HCV may increase the risk of end-stage liver disease is still uncertain [227]. Reactivation of schistosomiasis after liver transplantation (with intestinal tract and graft involvement) although infrequent, may occur, but praziquantel achieved cure in published cases [228]. Close post-transplant monitoring is recommended.

44.2.2.3 Infected Donors

Schistosoma infections are often subclinical, and patients may be unaware of the infection. While schistosomes can be transmitted by the graft, adult schistosomes do not replicate within the host so only transmission of nonreplicating adult worms occurs [149]. Liver grafts infected with *S. mansoni* (from deceased and living donors with undiagnosed infection) have been incidentally transplanted [228–230].

Intentional use of infected grafts has also been reported [231]. Praziquantel treatment after transplantation eradicated the parasite disease in recipient and living donors without further consequences.

Kidneys without anatomical damage from infected living donors have been accepted for donation [232]. Treatment should be given to all infected donors before surgery. Kidney function and morphology are not affected by nephrectomy in infected donors on long-term follow-up [233].

44.2.2.4 Diagnosis

The diagnosis is made by detection of eggs in stool or urine samples, or bladder and rectal mucosa biopsy specimens. False negative may occur in low-burden or initial infection.

Serological assays have limited usefulness, as they do not correlate with parasitic load, and seroconversion may occur several months after primary infection.

44.2.2.5 Treatment

Praziquantel is effective against the five *Schistosoma* species that infect humans. Dosing for *S. haematobium, S. mansoni,* or *S. intercalatum* is 40 mg/kg (in one or two divided doses). Higher doses should be used for *S. japonicum* or *S. mekongi* (60 mg/kg in two divided doses) [234]. Cure is achieved with the first course of treatment, even in chronic disease, in more than 60 % of patients. In those who remain infected, cure is usually achieved with a second course. Oxamniquine may also be effective. Praziquantel is a substrate of CYP3A4 [235] which is also a cyclosporine metabolic pathway. Close monitoring is warranted when cyclosporine and praziquantel are concurrently used. Potential interactions with other immunosuppressive agents have not been noted [148].

44.2.2.6 Prevention

Disease prevention involves avoiding contact with fresh water in endemic regions and screening and treating positive donors and recipients.

44.2.3 Echinococcosis

Echinococcosis, a parasitic disease for which domestic or wild canids are definitive host, is caused in humans by accidental ingestion of eggs of the cestodes *E. granulosus, E. multilocularis*, or *E. vogeli*.

E. granulosus causes cystic echinococcosis (CE), or hydatid or unilocular cyst disease, while *E. multilocularis* and *E. vogeli* cause alveolar echinococcosis (AE) cyst disease.

The clinical manifestations of cystic echinococcosis are determined by the site, size, and condition of the cysts. The slowly growing echinococcal cyst often is tolerated well until it causes dysfunction because of its size (mass effect of the enlarging cyst) or from the leakage, rupture, or bacterial infection of the cyst [236]. Although the mechanism of infection with *E. multilocularis/vogeli* is the same as that encountered in hydatid disease, its natural course is completely different because larvae proliferate making alveolar

cysts grow indefinitely, and they always behave as slow-growing cancers that in all cases require wide surgical resections to avoid recurrence. Alveolar echinococcosis is a fatal disease without treatment (>90 % mortality at 10 years).

44.2.3.1 Diagnosis

Diagnosis of CE and AE is based on typical imaging findings combined with positive serology. Serologic tests are more reliable for diagnosis of *E. multilocularis* infection than for *E. granulosus* infection. A negative serology does not rule out CE [237].

44.2.4 Cystic Echinococcosis (CE)

44.2.4.1 The Infected Recipient in Waiting List

Whenever possible, CE should be recognized and treated with the surgical removal of the fertile cysts and albendazole before transplantation [238]. Successful "delayed" management after HSCT has been described [239]. Uncomplicated, sterile WHO cysts (CE4 and CE5) do not need intervention [240]. Terminal organ failure due to CE has successfully been treated with liver [241–244] and kidney transplantation [245]. Although albendazole treatment before and after transplantation seems the logical approach [246], no recurrence of CE was observed even in absence of specific antiparasitic treatment [243].

44.2.4.2 Post-transplant CE

Management of CE acquired after transplantation (reported in kidney recipients) is similar to management in immunocompetent host. Surgical resection [246] and prolonged albendazole treatment are indicated [247, 248]. The role of immunosuppression with CE development in SOT patients is still uncertain, although unusual courses have been observed in immunosuppressed patients [240, 248–250].

44.2.4.3 Donors Infected with CE

Donors from endemic areas may have unrecognized hydatid cysts found at the time of organ procurement. Livers with inactive, calcified hydatid cysts have successfully been used, after complete [251, 252] or partial cystectomy of the graft [253].

44.2.5 Alveolar Cyst Disease (AE)

Alveolar echinococcosis is similar to a slowly growing hepatobiliary cancer in its clinical behavior.

Liver transplantation may be indicated in cases with extensive liver involvement or complications. Patients should be evaluated for extrahepatic involvement before transplantation, as the disease may spread to lungs and brain. Only CNS involvement should be considered as exclusion criteria for the procedure.

Survival rates are similar to those observed in patients transplanted for other liver diseases [254]. Immunosuppression can enhance the parasitic growth and the risk of recurrence; therefore, it should be reduced to a minimum as early as possible. Treatment with albendazole (15 mg/kg/day) is recommended for a minimum of 2 years after transplantation even in cases of apparently curative surgery. ELISA measuring anti-*E. granulosus* immunoglobulin G titers is considered useful for predicting recurrence.

44.3 Free-Living Amebas

44.3.1 ***Balamuthia mandrillaris*** and ***Acanthamoeba*** Species

B. mandrillaris has been isolated from soil, dust, and water. *Acanthamoebae* species are also ubiquitous in the environment. Both microorganisms are acquired through inhalation or damaged skin and causes granulomatous amoebic encephalitis (GAE), a sub-acute life-threatening encephalitis. *Balamuthia* infection is rare (<200 cases reported worldwide), and seems to be associated with contact with soil or stagnant water [255]. *Acanthamoebae* species are the most common causes of GAE in immunocompromised hosts. GAE may be sometimes preceded by skin lesion (usually non-ulcerated plaques) and followed by CNS disease. *Balamuthia* GAE was reported in one multivisceral transplanted patient after alemtuzumab therapy [256] and transmission via organ donation has been described in three clusters, including one asymptomatic donor [257–259]. *Acanthamoeba* GAE has been reported in a few SOT [260] and HSCT [261–263] recipients.

In SOT recipients, *Acanthamoeba* GAE presentation occurred at median time of 13 months (range: 3–48) after transplantation [260]. It may cause focal disease (such as keratitis, skin, pulmonary or brain lesions, sinusitis, or GAE) or disseminated disease that most often involves the skin and lungs. After onset of neurologic symptoms, there is a rapid disease progression leading to death. Early diagnosis is crucial for survival.

44.3.1.1 Diagnosis

Diagnosis is made by histopathological examination of infected tissues. Definite diagnosis requires identification of trophozoites in biopsies which usually show granulomatous inflammation and necrosis. Direct immunofluorescence or immunoperoxidase staining and PCR techniques [264] are highly sensitive and species-specific. Occasionally, trophozoites may be observed in CSF, or CSF–PCR positivity may allow diagnosis.

Serological tests, while not useful in *Acanthamoeba* infections, may aid in *Balamuthia* diagnosis, and in monitoring transmission and therapy in SOT recipients [259].

Acanthamoeba can be cultured on agar plates coated with Gram-negative bacteria.

44.3.1.2 Treatment

Optimal treatment for *Balamuthia* infections is unknown. Combinations of macrolides, pentamidine, anti-fungal agents (amphotericin B, azoles, and 5-flucytosine), albendazole, sulfadiazine, and miltefosine, either for pre-emptive or established disease, are usually administered for a long period of time [255]. Serologic monitoring may be useful to decide treatment duration. Pre-emptive treatment to asymptomatic recipients of infected donors has proven to be successful [259], but in patients with established GAE, mortality is high in spite of aggressive therapy.

Ideal treatment for *Acanthamoeba* species is also unknown. Cure has been achieved with rifampin and trimethoprim–sulfamethoxazole (TMP-SMX) [265]. Successful therapeutic alternatives include TMP-SMX, rifampin, fluconazole, ketoconazole, miltefosine, and pentamidine. Single brain lesions resection is recommended regardless of GAE etiological agent [260, 266].

44.3.2 *Naegleria fowleri*

N. fowleri is found in contaminated warm freshwaters. It causes a fulminant primary amebic meningoencephalitis (PAM) that mimics acute bacterial meningitis. *N. fowleri* reach the brain through the olfactory tract, when contaminated water moves up to the nose, such as during swimming or nasal irrigation. Transmission of *N. fowleri* through organ transplantation was not observed in 21 recipients from five donors with PAM. However, the risk of transmission may still exist [267].

44.4 Intestinal Protozoa

44.4.1 Cryptosporidiosis

Cryptosporidium species have gained increasing recognition as some of the most common enteric parasitic pathogens in humans and as a worldwide cause of diarrheal diseases in normal hosts [268, 269]. *Cryptosporidium* infection in immunocompromised patients can be asymptomatic, can cause transient gastrointestinal symptoms, produce chronic diarrhea, or cause life-threatening gastrointestinal and biliary tract disease [270]. In endemic regions, prevalence of up to ~30 % has been reported in kidney transplant recipients [271–273]. *Cryptosporidium* organisms are intracellular protozoan parasites that develop in the gastrointestinal tract of vertebrates, producing gastrointestinal disease; they do not require extraintestinal development [274]. Transmission of the *Cryptosporidium* infection occurs by four major modes: (1) person to person, which is especially relevant among household members, sexual partners, children in day care centers and their caretakers, and health care workers [275, 276]; (2) animal to human, which, although infrequent, is a higher risk for persons in contact with farm animals than for those who have contact with pets [274, 277]; (3) contaminated food; and (4) fecally contaminated water, untreated surface water, filtered swimming pool water, and even chlorinated or filtered drinking water [278].

44.4.1.1 Cryptosporidiosis in Solid Organ Transplantation

Reports of cryptosporidiosis have been published in kidney [270–273, 279–282], liver [282–286], small bowel [287, 288], and HSCT [289–295] recipients.

Infection spread out of the gastrointestinal lumen is very rare: One case of lethal *Cryptosporidium baileyi* infection with disseminated infection has been reported [280] and a case of sclerosing cholangitis secondary to intestinal cryptosporidiosis has been accounted for [296] in a kidney transplanted patient. *Cryptosporidium*-associated sclerosing cholangitis is a rare but severe complication that has been reported in adults and in children with liver transplantation [283, 286].

In small bowel transplant recipients, *Cryptosporidium* has been described to cause severe diarrhea [284, 287, 288] that needs to be differentiated from rejection and from other infectious agents [287]. Cryptosporidiosis in solid organ recipients has been associated with elevated tacrolimus levels and a transient worsening of kidney function [282].

44.4.1.2 Cryptosporidiosis in Hematopoietic Stem Cell Transplantation

Cryptosporidium infection in HSCT recipients causes diarrhea (which may be severe) [289], vomiting, anorexia, and weight loss [295]. Extraintestinal cryptosporidiosis with pulmonary involvement [291] has been reported. Intestinal cryptosporidiosis can mimic intestinal graft-versus-host disease (GVHD) and needs to be differentiated from it, since clinical management requires different approaches [294].

44.4.1.3 Diagnosis

Cryptosporidiosis should be considered in all transplant recipients with acute or chronic diarrhea or signs of biliary tract disease without biliary calculi.

The diagnosis of *Cryptosporidium* infection is made primarily by oocysts detection in a modified stool acid-fast staining. Oocyst excretion is intermittent, so multiple sam-

ples may be necessary to reach diagnosis. IFA and ELISA assays can significantly increase diagnostic sensitivity to as high as 100 % [297]; these techniques have been used in fecal or tissue specimens [268]. When available, polymerase chain reaction (PCR) testing is the diagnostic method of choice [298]. Serologic testing has a limited value for clinical diagnosis because antibodies can be elevated for more than 1 year after acute infection. Duodenal aspirates and biopsy specimens from affected gastrointestinal tissue [274, 284] may be required to reveal the parasite. A definite diagnosis of biliary tract cryptosporidiosis is made by the demonstration of oocysts in the bile or by histology. Stool specimens may or may not be positive.

44.4.1.4 Treatment

No reliable treatment for cryptosporidiosis exists. The resolution of *Cryptosporidium* infection depends mainly on the immune status of the host. Although the available treatments do not reliably eradicate the parasite, they do seem to suppress the infection [297]. Long-term therapy, including nitazoxanide, azithromycin, spiramycin, or paromomycin, may be effective [299, 300]. However, it is unclear whether therapeutic success can be achieved without reducing immunosuppression [288, 301], as impaired cell-mediated immunity and interferon-gamma play a key role in parasite clearance [301].

44.4.1.5 Prevention

Cryptosporidiosis is difficult to prevent because the oocyst is resistant to most of the standard water treatments and antiseptic solutions. Transplant recipients should limit their exposure to *Cryptosporidium* pathogens by avoiding contact with (including drinking) water from public swimming pools, lakes, and streams. Boiling water for 1 min or filtering it with submicron personal-use water filters may decrease the risk of infection, and these have been suggested as preventive measures, especially during outbreaks [302]. Ice made from tap water may be a source of infection, and patients should be advised accordingly. Bottled or canned carbonated soft drinks and pasteurized beverages are safe [302].

44.4.2 Microsporidiosis

Microsporidia are obligate, spore-forming, intracellular, highly diverged parasites [303]. Microsporidia are ubiquitous, highly resistant to degradation, and have the ability to survive in the environment for many months; in addition, they can infect the whole animal kingdom. *Enterocytozoon bieneusi* and *Encephalitozoon* spp. are the most common causes of human disease [303].

Fecal–oral and urinary–oral routes seem to account for most infections, although person-to-person transmission is possible due to the presence of spores in body fluids. Microsporidiosis is a zoonotic disease [303]. Birds, many animals, and insects are known reservoirs for microsporidial species that produce human illness. Also, there is evidence of waterborne and foodborne transmission [303]. Transmission to multiple recipients from an infected organ donor has been reported [304].

E. bieneusi and *Encephalitozoon* spp. infect the gastrointestinal and biliary tracts. In general, *Enterocytozoon* infections are limited to the intestine producing chronic diarrhea and weight loss [305]. *Encephalitozoon* spp. infects macrophages and disseminates widely, causing systemic infection with involvement of the intestinal and hepatobiliary tracts, the respiratory tract, sinuses, eye, brain, and kidney [305]. *Anncaliia algerae* an insect microsporidia, and causes myositis in immunocompromised patients [306, 307].

44.4.2.1 Microsporidiosis in Transplantation

E. bieneusi asymptomatic carriage [308] and symptomatic infection have been reported in transplant recipients [309]. *E. bieneusi* invades the intestinal epithelium and may spread to the hepatobiliary canals, producing cholangitis. Symptomatic infection in kidney, liver, and heart–lung [310–316] recipients is characterized by non-bloody, watery, protracted diarrhea; fatigue; abdominal discomfort; weight loss and fever [317]. In spite of chronic diarrhea, reports indicate that the macroscopic appearance of the intestinal mucosa at the time of endoscopic studies is normal [310, 312, 314, 315], whereas duodenal biopsy specimens show that mild, nonspecific duodenitis [314, 315], and colonic biopsy specimens are usually normal.

***Encephalitozoon* spp.** causes disseminated disease with multiorgan involvement, graft malfunction [318], and pulmonary disease. Disseminated infection has been reported in HSCT (with pulmonary involvement and with parasites identified in respiratory specimens) [319, 320]; in kidney recipients [321] (with involvement of kidney allograft in all cases, with cornea, lungs, and central nervous system among possible sites of dissemination) [322]; in a lung recipient (with pulmonary involvement and granulomatous interstitial nephritis) [323]; and in kidney–pancreas transplant (with parasites identified in the abdominal fluid) [324].

A. algerae was reported to cause fever, myositis, and axonal neuropathy that was lethal in two transplanted patients [306, 307].

Disseminated lethal infection due to ***Tubulinosema acridophagus*** has been reported in a HSCT patient [325].

44.4.2.2 Diagnosis

Microsporidial infection diagnosis requires not only a high level of awareness but also trained laboratory personnel and specialized technical approaches.

The diagnosis depends on identification of spores in clinical samples that are obtained on the basis of patient symptoms but may include stool, urine, sputum, bronchoalveolar lavage fluid, and cerebrospinal fluid [326]. Due to intermittent oocyst shedding, multiple specimens of urine and stool may be necessary to ensure diagnosis [326]. Routine examination for ova and parasites will not detect microsporidia spores.

Specialized methods that include Ritchie concentration, Weber trichrome stain, and fluorochrome stain are needed for detection. In tissue, microsporidia stain variably with hematoxylin and eosin; Gram stain and Warthin–Starry techniques considerably improve the visualization of these organisms. Electron microscopy, molecular assays, or indirect immunofluorescence or immunohistochemical stains are usually needed for species-specific identification, which has important therapeutic implications [326].

44.4.2.3 Treatment

The successful management of microsporidial infections may require immunosuppression decrease whenever feasible, as cell-mediated immunity is paramount in controlling microsporidial infection [326].

Therapy for microsporidiosis is species-dependent. *Encephalitozoon* species, including *Encephalitozoon cuniculi*, are treatable with albendazole (400 mg twice daily) [326]. Survival rates may be close to 80 % when treatment is timely initiated, but prolonged therapy may be necessary.

E. bieneusi can be treated with oral fumagillin (20 mg three times a day given for up to 14 days) with early resolution of diarrhea [327]. Reversible thrombocytopenia is the most important toxicity, and platelet monitoring is necessary throughout treatment. Tacrolimus blood levels usually decrease with fumagillin treatment, so through levels monitoring is needed [327].

44.4.2.4 Prevention

Sensible precautions include the avoidance of known reservoir hosts, especially pets; thorough cooking of meat [328] and water boiling; and refraining from swimming in lakes, rivers, and swimming pools [329] as this is a risk factor for microsporidial infections [330].

44.4.3 Other Intestinal Protozoa

Diarrhea may occur in up to 20 % of solid organ transplant recipients [331] and close to 50 % of HSCT patients in developing countries. In many of these cases, intestinal protozoa, sometimes as mixed infections, are the responsible etiological agents of significant and protracted diarrhea. Although worldwide distributed, transmission is more common in developing areas. They share the same transmission mechanisms, related to contaminated food and water, person-to-person spread, and zoonotic exposures. Asymptomatic carriage may occur, and progression to symptomatic disease may occur when the patients are immunosuppressed.

In the HSCT setting, awareness of potential infectious causes of diarrhea is specially important after engraftment, as diarrhea may be considered as caused by drug toxicities or graft-versus-host disease.

*44.4.3.1 **Entamoeba histolytica***

E. histolytica has been reported in both SOT [332] and HSCT recipients [333] as a cause of invasive diarrhea. Skin involvement with erythema nodosum-like lesions caused by *E. histolytica* has been reported in a kidney transplant recipient [334].

Treatment involves the use of amoebicidal drugs (metronidazole or tinidazole) active against trophozoites (the invasive form) followed by a luminal agent to eliminate cyst (paromomycin or iodoquinol). Asymptomatic carriage can be treated with a luminal agent to prevent transmission and development of invasive disease.

*44.4.3.2 **Giardia lamblia***

G. lamblia is the most common intestinal protozoan parasite in the Western world; however, reports of these infections in the transplantation setting are scarce [335–337].

Giardia has been found to account for ~20 % of symptomatic parasitic diseases in kidney transplant recipients from South Brazil [338] and 27 % of all infectious diarrhea episodes in SOT recipients in Turkey [339]. Duodenal villous atrophy with diarrhea, usually caused by mycophenolic acid, may be caused by *Giardia* infection in ~10 % of the cases, and diagnosis may need upper gastrointestinal endoscopy because of negative stool examinations [340].

Giardia can be treated with metronidazole, nitazoxanide, or paromomycin. Refractory cases can be treated with metronidazole plus quinacrine.

*44.4.3.3 **Blastocystis hominis***

In kidney transplant recipients, gastrointestinal symptoms with detection of *B. hominis* in the stool samples have been reported; the prevalence of infection was nearly 40 % [272]. Among infected patients with no other enteropathogenic microorganism, 61 % were symptomatic, and symptoms correlated with the presence of amoeboid forms and parasitic load [272].

B. hominis infection has also been reported in an allogeneic HSCT recipient with acute bowel GVHD. Symptoms resolved only after long-term treatment with metronidazole cleared *B. hominis* from stools [341].

B. hominis can be treated with metronidazole (200–750 mg three times daily for 5–10 days). Cotrimoxazole and nitazoxanide can also be effective, but therapeutic failure can occur with all agents.

44.4.3.4 *Cyclospora cayetanensis* and *Cystoisospora belli*

C. belli has been reported as cause of profuse diarrhea in liver [342, 343], kidney [344, 345], and intestinal transplant [346] recipients. *C. cayetanensis* was also reported as cause of diarrhea in a kidney transplant recipient [347]. All cases resolved with trimethoprim/sulfamethoxazole, which may be used at 160 mg/800 mg dose twice or eventually four times daily.

44.4.3.5 *Diagnosis of Intestinal Protozoa*

Standard stool examination is useful to detect all above-mentioned intestinal protozoa. Concentration methods and special stains or techniques enhance detection of certain pathogens.

E. histolytica and *G. lamblia* detection is improved using species-specific antigen testing or PCR (stool microscopy does not allow differentiation between *Entamoeba* species). Serology does not distinguish past from present infection, and may be positive in asymptomatic individual. It may be useful in extraintestinal forms of amebiasis.

Cystoisospora and *C. cayetanensis* oocysts can be detected by modified acid-fast staining of stool and safranin stain. Fluorescence microscopy is useful to detect *Cyclospora* oocysts, which are autofluorescent.

PCR techniques, when available, are the most sensitive methods for detection.

44.4.3.6 *Prevention*

Routine parasitological screening should always be considered in the pretransplant work up for patients at epidemiological risk. Asymptomatic carriage should prompt enhanced efforts on patient education on food and water safety, hand washing, and hygiene. Pre-emptive treatment before starting immunosuppression may avoid development of symptomatic disease.

44.5 Conclusion

Parasitic disease epidemiology is changing. Their human hosts move around the world, carrying them; also there is an increasing number of people traveling [348]. Because of immigration, in 2013, 232 million people—3.2 % of the world's population—lived outside their country of origin [349]. Global warming and climate change are also modifying the geographical distribution of infectious diseases vectors [350–352] with the emergence of exotic infections in unexpected places [353]. The practice of infectious diseases in transplantation is always challenging, as clinical manifestations in patients with blunted immune responses are frequently subtle and atypical. Parasitic infections add to this challenge the need to be aware of their occurrence in unexpected geographical areas. Efforts should be focused on performing a comprehensive history, maintaining a high index of suspicion, and adhering to preventive measures. In the pretransplantation period, a thorough history of possible geographic exposure, even in the remote past, is essential; this needs to include the patient's areas of residence and travel. Inquiries about past infection should not be limited to their clinical manifestations, but they should also include the specific treatments that might have been administered even in the absence of clinical disease. In an attempt to prevent overwhelming infections after transplantation, serotesting and a parasitologic examination should be performed whenever reasonable doubt is present. The investigation should be carried out when patients are placed on the waiting list to allow time for diagnosis and pretransplantation treatment. Living donors should be thoroughly evaluated early in the pretransplantation workup to allow risk appraisal, diagnosis, and treatment as needed. Deceased donors pose an altogether different problem. Because an extensive epidemiologic history might prove difficult to obtain, the risk potential may be best evaluated by using laboratory tests more liberally.

In the immediate post-transplantation period, a high level of suspicion is needed because the symptoms of parasitic diseases might mimic those of other infections or even of rejection, especially when they present with fever and allograft dysfunction.

In the late post-transplantation period, recent exposure to endemic diseases needs to be addressed at the time of differential diagnosis.

Parasitic diseases may jeopardize transplantation success, especially if the diagnosis, specific treatment, and adequate management of immunosuppression are not accomplished in time.

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Part VIII
Infection Control

45 Infection Control Issues After Hematopoietic Stem Cell Transplantation

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45.1 Introduction

Preventing the acquisition and transmission of infections in hospitalized hematopoietic stem cell transplant (HSCT) recipients remains a challenge. Considerable clinical experience has been accumulated, but a paucity of randomized, controlled trials exists on the topic of infection control in this setting. The Center for International Blood and Marrow Transplant Research (CIBMTR), National Marrow Donor Program (NMDP), European Blood and Marrow Transplant (EBMT) Group, American Society for Blood and Marrow Transplant (ASBMT), Canadian Blood and Marrow Transplant Group (CBMTG), Infectious Diseases Society of America (IDSA), Society for Healthcare Epidemiology of America (SHEA), Association of Medical Microbiology and Infectious Diseases (AMMI), the Centers for Disease Control and Prevention (CDC), and the Health Resources and Services Administration published comprehensive evidence-based guidelines in 2009 (hereafter referred to as the "2009 Guidelines") for preventing opportunistic infections among HSCT recipients [1]. This report represents an update on earlier guidelines published in 2000 [2]. The 2009 Guidelines include 253 recommendations regarding hospital infection control practices in hematopoietic stem cell transplantation. The topics covered include room ventilation; construction, renovation, and building cleaning; isolation and barrier precautions; hand hygiene; equipment management; plants, play areas, and toys; issues involving health care workers (HCWs) and visitors to hematopoietic stem cell transplantation centers; patient skin and oral care; prevention of bacterial intravascular catheter-related infections; infection control surveillance; and prevention and control of specific nosocomial infections, including *Legionella* species, methicillin resistant *Staphylococcus aureus* (MRSA), Staphylococci with reduced susceptibility to vancomycin, vancomycin-resistant Enterococcus (VRE), multidrug-resistant gram-negative bacilli (MDR-GNB), *Clostridium difficile*, community respiratory viruses (CRVs), and viral gastroenteritis pathogens [1].

These 253 guidelines were stratified on the basis of the strength of the recommendations (A–E) and the quality of the supporting evidence (I–III). This document includes only 30 guidelines in category AI or II (strongly recommended) and two in category EII (never recommended) [1]. Table 45-1 summarizes the definitions of these categories and lists the 32 AI, AII, and EII guidelines.

In addition to the 2009 Guidelines, several general infection control policy guidelines are relevant to the care of the HSCT population. The most important of these for HSCT infection control are the "2007" Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Health Care Settings" of the Healthcare Infection Control Practices Advisory Committee (HICPAC) [3], the 2003 "Guidelines for Environmental Infection Control in Healthcare Facilities" [4] from HICPAC, the 2002 "Guideline for Hand Hygiene in Healthcare Settings" [5], and the 2008 "Guideline for Disinfection and Sterilization in Healthcare Facilities" [6]. Additional guidelines specific to a particular pathogen (i.e., *C. difficile,* norovirus) were published after the 2009 Guidelines and will be mentioned in the relevant sections [7, 8].

45.2 Guideline Recommendations

45.2.1 Room Ventilation

Ventilation is an extremely important infection control measure, but one in which practices have varied from one center to another. All allogeneic recipients should be placed in rooms with at least 12 air exchanges per hour and central or point-of-use high-efficiency particulate air (HEPA) filters that are capable of removing particles larger than 0.3 mm in diameter (AIII) [1, 9, 10]. The efficacy of these protective isolation measures for autologous HSCT recipients has not been well established (BIII). Effective filtration is particularly

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TABLE 45-1. AI, AII, and EII recommendations stratified by strength and quality of supporting evidence in the 2009 Guidelines, infection control section

Categories

A. Strong evidence for efficacy and substantial clinical benefit;
B. Strong or moderate evidence for efficacy but only limited clinical benefit;
C. Insufficient evidence for efficacy or efficacy does not outweigh possible adverse consequences or cost of chemoprophylaxis or alternative approaches;
D. Moderate evidence against efficacy or for an adverse outcome;
E. Strong evidence against efficacy or for an adverse outcome.

Strength of supporting evidence

I. Evidence from at least one well-executed randomized, controlled trial;
II. Evidence from at least one well-designed clinical trial without randomization, cohort, or case-controlled analytic studies (preferably from more than one center), multiple time-series studies, or dramatic results from uncontrolled experiments;
III. Evidence from opinions of respected authorities based on clinical experience, descriptive studies, or reports of expert committees.

AI recommendations

1. When hands are visibly dirty or soiled with blood or body fluids, hands should be washed using soap and water.
2. When a definite or possible case of laboratory-confirmed nosocomial Legionnaires disease (LD) is identified in a person who was in the inpatient HSCT center during all or part of the 2–10 days before illness onset, or if two or more cases of laboratory-confirmed LD occur among patients who had visited an outpatient HSCT center, the hospital infection control team should be consulted and a thorough epidemiological and environmental investigation should be conducted to determine the likely environmental source(s) of *Legionella* species.
3. HSCT centers should follow published recommendations for preventing, controlling, and treating *C. difficile* infection.
4. All HCWs who have contact with a *C. difficile* infected patient or with the patient's environment should don gloves.
5. HCWs and close contacts of HSCT recipients should receive a yearly influenza vaccine at the start of the influenza season, preferably with inactivated influenza vaccine rather than with live attenuated influenza vaccine to avoid concerns about transmission of vaccine virus.

AII recommendations

1. HSCT centers should prevent birds from nesting near hospital air-intake ducts.
2. Construction and renovation areas should have negative air pressure relative to HSCT patient care areas to ensure that air flows from patient care areas toward construction areas.
3. A portable, industrial-grade HEPA filter should be used between a construction zone and the HSCT unit if a large area is under construction and negative pressure differential cannot be guaranteed.
4. Hand hygiene is the mainstay of infection prevention in a hospital and is an essential element of Standard Precautions for all patients.
5. In the absence of visible soiling of hands or contact with spore-forming organisms, the preferred method of maintaining hand hygiene is use of an alcohol-based hand rub because of its superior microbicidal activity, reduced drying of the skin, and convenience.
6. Every effort should be made to restrict all HCWs with infections that are potentially transmissible to HSCT recipients or candidates from direct patient care activities.
7. Work-exclusion policies should be designed to encourage HCWs to report their illnesses or exposures.
8. Staff should request that visitors with CRV leave the HSCT center until signs and symptoms of infection have resolved or, for recent exposures to communicable infections, until the incubation period for that infection has passed without the appearance of signs or symptoms suggestive of active infection.
9. If *Legionella* species are detected in the water being supplied to a HSCT center, the water supply should be decontaminated until *Legionella* species are no longer detected by culture.
10–13. To prevent MRSA, VRE, MDR-GNB, and CRV transmission, HCWs should maintain hand hygiene with either an alcohol-based hand rub or wash hands with soap and water if soiled before and after all patient contact or contact with the patients' potentially contaminated equipment or environment.
14–16. To prevent MRSA, VRE, and MDR-GNB transmission, HCWs should use Contact Precautions for patients colonized or infected with these pathogens, including the use of gloves and gowns.
17. All HSCT centers should have sufficient laboratory capability to identify all Staphylococcus isolates and their antimicrobial susceptibility patterns, including vancomycin susceptibility.
18. Medical and ancillary staff members who are responsible for monitoring antimicrobial use patterns in the facility should routinely review vancomycin use patterns.
19. HSCT center personnel should institute the prudent use of all antimicrobials, including vancomycin, to prevent the emergence of Staphylococcus with reduced susceptibility to vancomycin.
20. To reduce the risk of VRE infection, HSCT clinicians should minimize the use and duration of treatment with vancomycin and antimicrobial agents with anaerobic coverage.
21. HCWs and visitors with infectious conjunctivitis should be restricted from direct patient contact until the drainage resolves.
22. HSCT recipients with CRV infection should be placed on appropriate precautions for at least the duration of illness.
23. HSCT centers should ensure adherence to hand hygiene, appropriate isolation precautions, and environmental disinfection when patients develop viral gastroenteritis.
24. When a patient has viral gastroenteritis, appropriate precautions should be maintained for at least the duration of illness.
25. Contact Precautions and environmental disinfection should be used to control the spread of astrovirus infection among HSCT recipients during known outbreaks.

EII recommendations

1. Treatment of asymptomatic *C. difficile* carriers to prevent clinical infection.
2. Administration of Bacillus Calmette–Guérin vaccination to HSCT recipients.

From Tomblyn M, Chiller T, Einsele H, et al. Guidelines for Preventing Infectious Complications among Hematopoietic Cell Transplantation Recipients: A Global Perspective. Biol Blood Marrow Transplant. 2009; 15: 1143–1238.

Abbreviations: *HSCT* hematopoietic stem cell transplant, *CRV* community respiratory virus, *HCW* health care worker, *MDR-GNB* multidrug-resistant gram-negative bacilli, *MRSA* methicillin-resistant *Staphylococcus aureus*, *VRE* vancomycin-resistant Enterococcus.

[a]See text for updates from more recent guidelines regarding these points.

important and should be monitored frequently in HSCT centers undergoing construction and renovation (AIII) [11, 12]. When there is a shortage of such rooms to accommodate all HSCT recipients, room allocation should prioritize those at highest risk for invasive mold infection (BIII). In such instances, portable, industrial-grade HEPA filters may be used in non-protective environment rooms to minimize risk to vulnerable patients (BIII). Though portable HEPA filters have been demonstrated to remove airborne fungal spores and mycobacteria, their efficacy relative to the standard central or point-of-use HEPA filters in preventing infection has not been established [13, 14].

Opinion has evolved regarding the utility of laminar air flow (LAF) rooms, in which HEPA-filtered air moves in parallel in one direction and exits from the wall opposite where the air enters. Early studies suggested decreased mortality with use of LAF rooms for BMT patients [15, 16]. However, more recent work has not shown an overall survival benefit from routine LAF use in all HSCT recipients [17, 18]. Due to their inconvenience, added expense, and lack of clear benefit, LAF rooms are not currently recommended for newly constructed rooms in HSCT centers (DII).

Current recommendations do suggest that rooms have directed airflow with air intake and air exhaust at opposite sides of the room (BIII) and that each room has well-sealed windows, electrical outlets, floor and ceiling (BIII). Hematopoietic stem cell transplantation centers should prevent the access of birds to hospital air intake ducts (AII) because studies have linked such exposures to the subsequent development of invasive pulmonary infections in HSCT recipients [9, 19]. In addition, a positive-pressure differential of ≥2.5 Pa should exist between the room and adjacent hallways or anterooms (BIII) [1, 10] and continuous pressure monitoring should be employed to allow staff to monitor for engineering failures (BIII) [4, 20]. This pressure differential decreases the likelihood of introduction of airborne pathogens from adjoining areas. Self-closing doors are useful in maintaining this pressure differential (BIII). Anterooms are optional, except in cases of HSCT recipients requiring Airborne Precautions (BIII). For the management of a patient requiring a protective environment who concurrently has an airborne infection when an airborne isolation room with an anteroom is not available, a portable, industrial-grade HEPA filter should be placed in the room to augment the removal of spores (BIII) [4].

Walsh and Dixon summarized the investigations and point sources found in 25 outbreaks of nosocomial aspergillosis [19]. Most commonly, aspergillosis outbreaks in neutropenic patients have been related to hospital construction or contaminated ventilation systems [19]. The authors recommended environmental microbiologic surveillance and floor-to-ceiling barriers during hospital construction and renovation as important measures for aspergillosis control [19]. In another study, an air pressure differential between an oncology unit and the adjoining hospital areas was thought to be the source of an *Aspergillus* outbreak during hospital construction [21]. Although the room pressure was higher than the corridor pressure in the oncology unit, the unit as a whole had negative pressure with respect to other hospital areas [21]. This case illustrates the complexity of the investigations required for aspergillosis control.

Even when the systems conform to the above specifications, at times these systems may be nonfunctioning or shut down for maintenance. The 2009 Guidelines recommend the establishment of backup systems for power and air-handling in the event of a shutdown of the central ventilation system (BIII). In addition, provisions should be made for the prevention of exposure to fungal spores after air-handling systems are restarted following routine maintenance (BIII).

The 2007 Guideline for Isolation Precautions develops further the idea of a "protective environment" for HSCT recipients [3]. The elements of such an environment include "(i) HEPA filtration of incoming air, (ii) directed room air flow, (iii) positive air pressure relative to the corridor, (iv) well sealed rooms (including sealed walls, floors, ceilings, windows, electrical outlets) to prevent flow of air from the outside, (v) ventilation to provide more than 12 air changes per hour, (vi) strategies to minimize dust (i.e., scrubbable surfaces rather than upholstery and carpet, and routinely cleaning crevices and sprinkler heads), and (vii) prohibiting dried and fresh flowers and potted plants in the rooms of HSCT patients" [3]. More detailed recommendations are listed in Table 5 of the 2007 Guideline for Isolation Precautions [3]. It is important to note that this concept of a "protective environment" does not include barrier precautions beyond routine application of Standard Precautions for all patients and Transmission-Based Precautions when indicated. In addition, evidence is lacking to recommend this protective environment to immunocompromised patients other than HSCT recipients [3]. Moreover, the use of HEPA-filtered rooms alone is not enough to prevent infections and should not replace other standard infection control measures, nor create a false sense of security. In a study of 23 outbreaks of viral, fungal, and bacterial infection in HSCT units, the majority occurred despite the use of HEPA-filtered rooms [22].

45.2.2 Construction, Renovation, and Building Cleaning

The major concern with hospital construction or renovation is the increased risk of aspergillosis and other fungal infections due to the aerosolization of fungal spores during construction activity. Close collaboration and advance planning by a multidisciplinary team involving infection control personnel and individuals responsible for construction, are important [4, 23]. Published guidelines for environmental controls during construction should be consulted during the planning and implementation of construction (AIII) [4, 23].

Education of construction personnel and HCWs can help minimize infection control risks and mandatory infection control adherence agreements should be incorporated into construction contracts [4]. Construction of new facilities or renovation of existing facilities offers an opportunity to maximize patient safety by adherence to guidelines [4]. For example, regarding air intake and exhaust outlets, building design should ensure that exhaust outlets are located atleast 25 feet from intake systems; that outdoor air intakes are located atleast 6 feet above ground or atleast 3 feet above roof level; and that exhaust outlets from contaminated areas are located above roof level to minimize recirculation of exhausted air [4].

In addition, in anticipation of construction or renovation, aspergillosis-control measures (AIII) should be intensified. Recommended measures include the placement of airtight barriers between patient care areas and construction areas (BIII) [4]; limiting the opening and closing of doors or other barriers (BIII) [4, 24]; the use of dedicated entrances, stairways, and/or elevators for construction traffic; and limiting contact between construction personnel and patient care areas by controlling the flow of patients during the construction [4]. It is preferable for air exhaust from construction areas to be vented outside the hospital rather than recirculated (BIII); if that is not possible, the exhaust should be HEPA-filtered. In addition, construction and renovation areas should have negative pressures relative to HSCT patient care areas to ensure air does not flow from the construction areas to the patient care areas (AII) [21, 25]. If this pressure differential cannot be guaranteed and a large area is under construction, a portable, industrial-grade HEPA filter should be used between these two areas (AII) [21, 26]. Measures should be in place to avoid tracking of dust and construction debris out of the construction zone, such as the use of mats with tacky surfaces, damp-wiping tools, and removal of workers' protective outer clothing when leaving the construction zone. Regular monitoring of compliance is essential [4]. Any visible dust and debris tracked out of the construction zone should be removed using a HEPA-filtered vacuum [27]. The 2009 Guidelines [1] and the 2003 Guidelines for Environmental Infection Control [4] present more detailed discussions of specific measures and should be consulted prior to such projects.

HSCT recipients, HCWs, and visitors should avoid construction and renovation areas to the extent possible, and HSCT equipment and supplies should not be exposed to construction areas (AIII) [21, 28]. The use of N95 respirators during transport has been advocated for HSCT recipients passing near construction or renovation areas (CIII) [21, 28]. Training in proper use is necessary for maximal benefit. The routine use of N95 respirators by patients, however, has not been evaluated for preventing exposure to fungal spores during periods of non-construction [4]. Standard surgical masks provide little protection against the inhalation of fungal spores and should not be used.

There should be enhanced monitoring for clinical cases of aspergillosis and other invasive mold infections in order to identify trends that may suggest an environmental mold source (BIII). That said, the utility of routine microbiological air sampling for fungal spores in HSCT units has not been established and is not recommended (DIII) [29–31]. There may, however, be a role for such testing during suspected outbreaks to determine the environmental source (CIII).

Newly constructed areas should be cleaned before patients are permitted to enter them (AIII), as several guidelines outline [4, 23]. With regard to hospital cleaning, HSCT patient care areas should be cleaned at least daily using an Environmental Protection Agency (EPA)-registered disinfectant (BIII). Particular attention should be given to dust removal (BIII). Wet dusting methods should be employed to avoid dust aerosolization (BIII) [4]. Floor surfaces and furnishings should be smooth, non-porous, and easily disinfected to minimize dust levels and contamination with nosocomial pathogens (BIII) [4]. Carpeting should not be installed in HSCT patient care areas, as contaminated carpeting has been associated with outbreaks of aspergillosis in HSCT centers [32, 33]. HSCT recipients should not be exposed to activities such as vacuuming that may result in the aerosolization of *Aspergillus* spores (EIII) [23]. Vacuum cleaners should be fitted with HEPA filters [4, 25]. To avoid mold contamination, water leaks should be repaired as quickly as possible and ideally within 72 h (BIII) [4, 34].

45.2.3 Isolation and Barrier Precautions

Hematopoietic stem cell transplantation centers should follow the 2007 Guidelines for Isolation Precautions [3]. The concept of a protective environment has been discussed above, with regards to airflow and ventilation considerations [3]. Since the early years of transplantation, the trend has been away from strict longterm protective isolation for all transplant recipients, with the recognition that many infections are due to host flora rather than acquired via person-to-person spread [35, 36]. In addition, the psychological effects of protracted strict isolation should be taken into account [35, 36]. Hand hygiene is the most effective preventive measure studied (see "Hand Hygiene"). One study indicated that gown and glove precautions appeared to have a protective effect in addition to that of strict hand washing when they were used in a pediatric intensive care unit dedicated to solid organ transplant recipients [37]; however, no recommendations to implement these precautions for all HSCT recipients exist. Additional precautions recommended for the control of specific organisms, such as multidrug resistant bacteria, are discussed in "Control of Specific Nosocomial and Community-Acquired Pathogens."

HSCT recipients should be housed in single-patient rooms (BIII). If the availability of such rooms is limited, priority should be given to the most immunosuppressed patients,

including HSCT recipients during their initial transplant admission—especially allogeneic transplants—and patients receiving therapy for graft-versus-host-disease (GVHD) during subsequent admissions (BIII).

The 2007 Guidelines for Isolation Precautions update previous guidelines in a number of important ways [3]. Precautions are now defined by a two-tiered system composed of Standard Precautions and Transmission-Based Precautions. Standard Precautions are used for the care of all patients in health care settings, and include the following [3]:

- Hand hygiene
- Safe injection practices
- Use of gowns, gloves, masks, eye protection, and face shields depending on the type of anticipated exposure
- Respiratory hygiene/cough etiquette

Respiratory hygiene/cough etiquette includes a variety of measures, such as education of health care providers, patients, and visitors; posted signs; source control measures such as covering the mouth and nose with a tissue when coughing; hand hygiene after contact with respiratory secretions; spatial separation, ideally more than 3 feet in waiting areas, and placing masks on coughing patients if tolerated [3]. Health care personnel with respiratory infection symptoms should avoid direct patient contact; if such contact is unavoidable, then a mask should be worn while providing patient care [3]. If specific respiratory viruses are diagnosed or suspected in hospitalized patients, however, Transmission-Based Precautions should be instituted (see below).

Transmission-Based Precautions are tailored to specific infections that a patient is known or suspected to have [3], and are designed to prevent the spread of pathogens to other patients by preventing health care personnel-associated spread. Compliance is particularly important in the HSCT population, as HSCT recipients with respiratory and gastrointestinal viruses can experience prolonged shedding of organisms. The three categories of Transmission-Based Precautions are Contact, Droplet, and Airborne Precautions; utilization of these precautions depending on the mode of spread of the known or suspected pathogen [3]. In some cases, more than one type of Transmission-Based Precautions may be applied, as when awaiting the results of a respiratory viral panel that includes agents spread by either droplet or contact or both. Contact Precautions involve donning gowns and gloves upon entry into the patient's room and removing these just prior to leaving, followed by hand hygiene. Examples of infections handled with Contact Precautions include *C. difficile*-associated diarrhea (CDAD), norovirus, and VRE [3]. Droplet Precautions are used for pathogens spread by close respiratory or mucous membrane contact, and involve health care personnel donning a mask prior to room entry, and patients wearing a mask when traveling outside the room, for example, for radiography and tests [3]. Examples of pathogens handled with Droplet Precautions include influenza virus, adenovirus, and *Neisseria meningitidis* [3]. Finally, Airborne Precautions are used for agents that remain infectious over long distances when suspended in the air, including measles virus, varicella virus, and *Mycobacterium tuberculosis* [3]. These precautions involve the use of airborne infection isolation rooms whenever possible. Such rooms have negative air pressure relative to the surrounding area, and have 6 exchanges of air per hour for existing facilities and 12 exchanges of air per hour for new construction, with air exhausted directly to the outside or HEPA-filtered prior to return [3]. Health care personnel don a respirator prior to room entry [3]. In settings where Airborne Precautions cannot be implemented due to limited engineering resources (i.e., physician offices), masking the patient, placing the patient in a private room (i.e., office examination room) with the door closed, and providing N95 or higher level respirators (or masks if respirators are not available) for health care personnel will reduce the likelihood of airborne transmission until the patient is either transferred to a facility with an airborne infection isolation room or returned to the home environment. Appendix A of the 2007 Guideline for Isolation Precautions contains detailed information on the type and duration of precautions for each infectious agent. An infection control practitioner should be consulted regarding any questions about which precautions to apply or when it is safe to discontinue precautions.

Meticulous hand hygiene should be taught to patients. Some clinicians recommend that HSCT recipients wear a surgical mask or N95 respirator and gloves when they are outside their hospital room prior to engraftment, but the poor utility of disposable surgical masks for prevention and inhalation of fungal spores calls this recommendation into question (CIII). Time spent in crowded areas of the hospital, such as waiting rooms and radiology suites, should be minimized to avoid exposure to respiratory viruses (BIII).

The SHEA 2015 Guidelines for Isolation Precautions for Visitors specifically address the necessity of hospital visitor adherence to isolation precautions [38]. These guidelines, which are not specific for HSCT recipients, suggest that the use of isolation precautions among visitors be guided by the specific pathogen, the underlying infectious condition, and the endemicity of the pathogen in both the hospital and community. Compliance with hand hygiene is essential for visitors of all patients, especially those on isolation precautions. In general, the use of gown and gloves by visitors of patients on Contact Precautions for MRSA and VRE is *not* recommended in these new guidelines, since these organisms are prevalent in the community [38]. However, special consideration should be given for visitors who are immunosuppressed themselves, who are unable to practice good hand hygiene, or who interact with multiple patients. Frequent visitors of patients on Contact Precautions during prolonged hospitalizations may also benefit from the use of Contact Precautions because there are likely to be more interactions that could result in a

higher likelihood of transmission to the visitor. Furthermore, Contact Precautions are recommended for visitors of patients infected with enteric pathogens (i.e., *C. difficile* and norovirus) and strong consideration should be given to their implementation for visitors of patients with drug resistant GNB, since these pathogens are not widely prevalent in the community [38]. Surgical masks are recommended for visitors to patients on both Droplet and Airborne Precautions [38]. Visitors with extensive exposure to a symptomatic patient prior to hospitalization, such as household contacts, may be excluded from these precautions since they may already be immune to the pathogen or in the incubation period [38]. The application of these 2015 Guidelines to HSCT centers is yet to be seen, since this vulnerable patient population frequently experiences prolonged hospitalization, often complicated by acquisition of multidrug resistant organisms (MDRO), enteric pathogens, and other infections requiring Transmission-Based Precautions.

45.2.4 Hand Hygiene

Good hand hygiene practice in the care of HSCT recipients is of paramount importance (AII); the 2002 Guideline for Hand Hygiene in Health Care Settings updates previous recommendations [5]. Hand hygiene leads to a reduction in the carriage of potential pathogens on the hands. Multiple studies report that it reduces morbidity and mortality from health care-associated infections [5]. All persons, especially HCWs, should perform hand hygiene before entering and after leaving patient rooms. Hospital policies should encourage visitors to maintain hand hygiene before and after patient visits (BIII). In addition, HSCT recipients and their household contacts should be educated about the importance of maintaining good hand hygiene both in the hospital and following discharge (BIII).

Hand hygiene should be performed in the following situations: before direct contact with patients; after contact with blood, body fluid, mucous membranes, non-intact skin, or wound dressing; before and after touching an invasive medical device; after contact with a patient's skin; when hands will be moving from a contaminated to a clean body site during patient care; after contact with medical equipment or elements of the environment close to the patient, and before donning and after doffing of gloves. Gloving is not a substitute for hand hygiene and should be performed in addition to hand hygiene where indicated.

Hand hygiene should be performed either with plain or antimicrobial soap and water or with alcohol-based hand rubs. Use of the latter is preferred due to their superior microbicidal activity, reduced drying of the skin, and convenience (AII). However, washing with soap and water is preferred when there is visible soiling of the skin with blood or body fluids or contact with norovirus or spore forming organisms, such as *C. difficile* (AI). Care should be taken to ensure that hand washing is performed with the intended cleansing substance, as one outbreak of *Stenotrophomonas* infection in a transplantation unit was traced to a HCW who used moisturizer rather than hand washing soap due to faulty replacement of hand soap [39].

HCWs engaging in direct patient contact should not wear artificial nails or extenders because they have been associated with outbreaks of gram-negative bacilli and candidal infections (EIII). One study reported that 86% of HCWs with artificial nails carried gram-negative bacteria, *S. aureus*, or yeast, compared with 35% of those without artificial nails before hand washing with soap [40]. In this study, only 11% and 38% of those with artificial nails cleared pathogens with soap washing or with an alcohol-based gel, respectively, compared with 14% and 80%, respectively, of those without artificial nails [40]. Another study reported an association between artificial nails and long natural nails and a prolonged outbreak of *Pseudomonas* infection in a neonatal intensive care unit [41]. Educational campaigns encouraging short natural nails and meticulous nail hygiene should be conducted among HCWs who have contact with HSCT recipients. The 2002 Guideline for Hand Hygiene in Health Care Settings recommends keeping natural nail tips less than ¼ inch long [5].

45.2.5 Equipments, Plants, Play Areas, and Toys

HSCT centers should adhere to established guidelines for the cleaning, sterilization, disinfection, and maintenance of devices and equipment, and they should use only FDA- and EPA-registered agents (AIII) [6]. Although not required by current guidelines, some clinicians recommend wiping the stethoscope head with a sterile alcohol wipe between patients to reduce the potential for bacterial contamination [42, 43]. Sterile bandages and wound dressings should be discarded if they are beyond the expiration date, if the packaging is damaged, or if visible contamination by moisture or other substances has occurred (BIII). Arm boards, if they are used, should have only sterile dressing materials, which should be changed frequently (BIII). Non-sterile tongue depressors should not be used as splints for catheter sites because they have been associated with outbreaks of fatal invasive *Rhizopus* infection (DII) [44].

Although exposure to plants and flowers has not conclusively been shown to cause infection, guidelines discourage having plants, dried, or fresh flowers in HSCT units or areas caring for HSCT patients. The rationale for this prohibition is the isolation of *Aspergillus* species from the soil of potted plants, the surface of dried flower arrangements, and fresh flowers [19, 45, 46]. In addition, high colony counts of gram-negative bacteria, especially *Pseudomonas*, have been identified in vase water of cut flowers [4]. HSCT candidates and recipients should be instructed to avoid contact with soil-based materials such as clay and potting soil in order to minimize their risk of mold infection (DIII).

For pediatric centers, the 2009 Guidelines contain a detailed section on the maintenance of hygiene for toys, games, videos, and play areas [1]. Toys should only be used if they are disposable or if they can be subjected to frequent cleaning, including laundering in the hot cycle of a washing machine, dry cleaning, or washing with an approved disinfectant. In one instance, a rotavirus outbreak was traced to playroom toys that were not disinfected according to protocol [47].

The 2008 Guideline for Disinfection and Sterilization in Healthcare Facilities has many specific recommendations regarding disinfection and sterilization of hospital items [6]. This document uses the common Spaulding classification method of dividing items used in patient care into critical (objects that enter sterile tissue or the vascular system, such as surgical instruments and cardiac catheters), semicritical (objects that contact mucous membranes or non-intact skin, such as endoscopes and respiratory therapy equipment), and noncritical (objects that may contact intact skin but not mucous membranes, such as blood pressure cuffs, crutches, and computers) [6]. In addition, this guideline contains detailed descriptions of different chemical disinfection agents and sterilization methods, including those for multidrug resistant bacteria [6].

45.2.6 Animals

HSCT recipients may come into contact with animals in health care facilities. These may be service animals, therapy animals, or personal pets that visit. The 2009 Guidelines address infection risks posed by animal contact in the home, but no recommendations are given regarding animal contact in health care facilities. In 2015 SHEA published expert guidelines regarding animals in health care facilities [48]. A review of hospital policies in this guideline revealed that 30% of hospitals surveyed excluded immunocompromised patients from animal-assisted activities and 46% from personal pet visitation. This guideline makes the recommendation that, in general, service animals, animal-assisted activities, and personal pet visitation should not be permitted for patients who are significantly immunocompromised, including HSCT recipients, or for those with isolation precautions [48]. These guidelines are not evidence-based, but rather, are a set of practical, expert opinion-based recommendations made in the absence of robust evidence to support practice.

45.2.7 Health Care Workers

HCWs represent an important reservoir of infections that may be transmitted to immunocompromised patients. An ailment that may seem minor to the HCW may pose a significant threat to the HSCT recipient. Any HCW with a transmissible infection should not have direct contact with HSCT recipients (AII). The type of work restriction imposed—work leave vs. temporary reassignment to nonpatient care duties—depends on the specific infection. HCWs with draining skin and soft tissue infections or other lesions involving the skin or mucous membranes (i.e., herpes simplex virus lesions) that cannot be completely covered should be restricted from patient care activities (BIII). HSCT center HCWs with blood-borne pathogens—including HIV, Hepatitis B or C viruses—should not be restricted from patient contact (DIII) [49, 50]. HSCT centers should follow published guidelines regarding the duration of work restriction (BIII) [51, 52] and should formulate work-exclusion policies to ensure that HCWs report illnesses or potential exposures to communicable pathogens (AII) and to make certain that these workers are not penalized for work absences. In the current climate of personnel shortages, applying no pressure, whether direct or indirect, to HCWs to continue working during these illnesses is particularly important. This is an important aspect of an institutional culture of safety [3].

Immunizations of HCWs are of tremendous importance in protecting vulnerable patients. HSCT centers should have written policies regarding HCW immunizations that meet guidelines recommended by the CDC, the Advisory Committee on Immunization Practices, and the Healthcare Infection Control Practices Advisory Committee (BIII) [53]. If possible, HCWs in HSCT units should preferentially receive inactivated vaccines rather than live vaccines to minimize the potential risk of transmission of vaccine virus to HSCT recipients (i.e., inactivated influenza) (AIII).

Influenza vaccination is a particularly important focus of prevention. HSCT recipients may not mount an effective antibody response to the influenza vaccine until at least 1 year after transplantation or longer in those with GVHD. To protect patients, HCWs should receive yearly influenza immunization [54]. Some acute care centers have implemented mandatory vaccination policies for HCWs as a means to ensure high vaccination rates and promote patient safety. Unfortunately, considerable mythology surrounds the influenza vaccine in general public opinion. HSCT centers should mount educational campaigns emphasizing the value of influenza vaccination for HCWs and family members of patients. These campaigns should stress the low risk of adverse effects from the vaccine and the fact that relatively minor influenza symptoms in the HCW or family member could translate into respiratory failure for HSCT recipients. The availability of antiviral agents for postexposure prophylaxis should not be cited as a reason for HCWs to avoid influenza vaccination [55].

Varicella immunization for VZV-seronegative HCWs is also important. Although approximately 90% of adults are VZV-seropositive, seronegative HCWs are at risk of infection, and can transmit VZV before the onset of the typical rash. Similarly, VZV-seronegative household contacts of HSCT recipients should be vaccinated to protect the patient from exposure to acute varicella. Ideally, this should occur at least 4 weeks before the start of the conditioning regimen, since the vaccine is a live-attenuated virus strain [2, 56].

In general, educational programs for health care personnel about the importance of vaccines have been found to increase compliance with best practices [3] and contribute to patient safety overall. It is desirable to implement formal educational programs for all personnel with direct patient contact.

45.2.8 Visitors to Transplant Centers

Visitors with signs or symptoms suggestive of communicable infections or recent known exposure to communicable infections should be prohibited from direct contact with HSCT recipients or candidates (BII). Visitors should be asked to return only after signs and symptoms of infection have resolved or, for recent exposures to communicable infections, until the incubation period for that infection has passed (AII). The 2009 Guidelines recommend that all visitors to HSCT centers adhere to recommended hand hygiene guidelines and isolation precautions regardless of age (AIII). However, though the more recently published SHEA 2015 Guidelines for Isolation Precautions for Visitors differ in their recommendations, and stratify the need for visitor compliance with isolation precautions by pathogen, infectious condition, and prevalence of the pathogen in the community (see Sect. 45.2.3 for more details) [38]. The number of visitors permitted at one time should be limited to a number that permits the nursing staff to perform infection screening and adequate instruction and supervision of hand hygiene and isolation precautions, when appropriate (BIII). HSCT centers should have written policies concerning screening of visitors for potentially communicable diseases (BIII). At some institutions, trained personnel perform active screening of all visitors to HSCT centers. If this is not feasible, signs should be posted to inform visitors about visitation restrictions. In addition to educational programs for health care personnel, education of family members and visitors can make them partners in patient safety by helping to ensure that all who enter the room are utilizing best practices for hand hygiene and other procedures relevant to infection control.

45.3 Patient Skin and Oral Care

The oropharynx is an important source of potential pathogens during high-dose chemotherapy and transplantation, particularly when significant mucositis develops [57]. In particular, viridans streptococci may cause a devastating syndrome of sepsis and respiratory failure, which may be associated with antimicrobial prophylaxis, including fluoroquinolones [58–60]. Other streptococci, *Capnocytophaga* species, anaerobes, yeast, and gram-negative bacilli can originate in the oropharynx and produce infection. Education regarding the importance of good oral and dental hygiene for at least the first year following HSCT is recommended to minimize the risk of oral infections (AIII). If time allows, HSCT candidates should undergo evaluation for dental disease before transplantation (AIII) with the elimination of likely sources of infection (BIII), which may require restoration of teeth, repair of ill-fitting dental prostheses, and extraction of compromised teeth [61, 62]. Ideally, 10–14 days should elapse between the completion of dental therapy and the initiation of a preparative regimen to enable adequate healing (AIII) [62]. Use of keratinocyte growth factor-1, palifermin, can be considered in order to reduce the incidence, severity, and duration of mucositis, the incidence of febrile neutropenia, and possibly bacteremia after HSCT [63].

Oral hygiene should be maintained in HSCT recipients with the use of four to six oral rinses per day with normal saline, sterile water, or sodium bicarbonate solutions (AIIII) [64, 65]. Some centers also use chlorhexidine rinses to decrease oral soft tissue disease and microbial burden [66]; others add oral topical antifungal agents, such as nystatin, in an attempt to decrease candidal colonization. HSCT recipients and candidates should brush their teeth two to three times per day with either a soft toothbrush that is regularly replaced or a foam tooth swab if use of a toothbrush is intolerable, though the latter is less effective (CIII) [62]. Patients skilled at dental flossing may continue to floss daily as long this can be done without inducing mucosal trauma (BIII). HSCT patients should not wear fixed orthodontic appliances from the start of conditioning until mucositis resolves in order to decrease the risk of mechanical trauma and oral infection (EIII) [62]. Use of removable dentures should be minimized during conditioning and the early post-transplantation period; if used, they should be worn while eating, cleaned twice daily, and soaked in an antimicrobial solution that is changed daily (BIII) [62]. Patients with GVHD involving the oral cavity should undergo frequent dental evaluations, as dental caries can develop more quickly in this population (BIII) [67]. Regular brushing, flossing, rinsing, fluoride treatments, and management of xerostomia are essential elements of oral care for patients with GVHD (BIII) [67].

Maintenance of good skin care is equally important in patients undergoing HSCT. HSCT recipients should take showers or baths using a mild soap daily during and after transplantation (BIII). Patients with GVHD involving the skin should regularly moisturize dry, intact skin in order to decrease pruritus and maintain skin integrity [68]. However, HSCT clinicians should also be aware that an outbreak of *Paecilomyces lilacinus* infection was traced to a contaminated, commercially available skin lotion [69]. Daily inspection of the entire skin by a HCW should be encouraged, because prompt treatment of any breach in skin integrity may prevent the development of serious infection. Particular attention should be paid to sites that can serve as portals of infection, including intravascular access sites and the perineum (BII). During catheter care, any drainage, erythema, or tenderness should be immediately reported by the nursing staff; this plays a crucial role in the reduction of catheter-associated infections [70]. The use of razors,

haircutting scissors, or other sharp objects in the hospital room should be prohibited. When clipping of fingernails and toenails is needed, this should be performed carefully, preferably before transplantation, or by a HCW if after transplantation. The feet should be covered with soft booties to avoid injuries while barefoot and to prevent shoe-related chafing. When wound or other dressings are necessary, the dressings should be designed, to the extent possible, so that tape is applied only to the dressing surface and not to the patient's skin.

HSCT recipients and candidates undergoing conditioning therapy should maintain good perineal hygiene to minimize loss of skin integrity (BIII). Patients should perform gentle but thorough perineal cleaning after each bowel movement and thorough perineal drying after each episode of micturition. Following urination or defecation, female HSCT recipients should wipe the perineum from front to back to prevent urethral contamination (AIII). To prevent vaginal irritation that can lead to mucosal breakdown, menstruating HSCT recipients should not use tampons (DIII). The use of rectal thermometers, suppositories, enemas, internal rectal exams, and penetrating anal intercourse should all be avoided in HSCT recipients to prevent mucosal trauma (DIII). In addition, diarrhea is common in patients receiving conditioning chemotherapy regimens, and perianal irritation leading to skin erosions, perianal abscesses, and bacteremia is a constant risk. Centers should develop protocols for the care of perianal skin and the prevention of skin breakdown with the use of a topical protective paste or ointment; supervision of cleansing after defecation may help to avoid complications.

45.4 Prevention of Intravascular Catheter-Related Infections

Prolonged multilumen intravenous access is a necessity during the transplantation admission for infusion of stem cells and the administration of chemotherapy, intravenous fluids, blood products, antibiotics, and sometimes parenteral nutrition. Before transplantation, a large-bore pheresis catheter may be used for stem cell harvesting in the case of autologous HSCT recipients, and certain types of catheters may sometimes be used both for pheresis and during the transplantation admission [71]. Protocols for catheter care differ considerably from one institution to another [72].

Catheter-associated blood stream infections (CLABSI) are a leading cause of bloodstream infections in HSCT recipients, particularly during the preengraftment phase and in patients with GVHD [1]. HSCT centers should follow published guidelines for preventing intravascular device-related infections (AIII) [73, 74]. In 2014 SHEA/IDSA published an update to the 2008 Strategies to Prevent Central Line-Associated Bloodstream Infections in Acute Care Hospitals [75, 76]. The recommendations are divided into basic practices that should be adopted in all acute care areas prior to insertion, at insertion, and after insertion of intravascular catheters. Prevention practices before insertion include: providing clinicians with easy access to an evidence-based list of indications for Central Venous Catheter (CVC) use; requiring education about CLABSI prevention for all health care personnel involved in insertion, care, and maintenance of CVCs; and daily bathing with a chlorhexidine preparation for ICU patients over 2 months of age. The role of chlorhexidine bathing in non-ICU patients remains to be determined. At the time of insertion the following prevention practices are recommended: require an insertion checklist (or other process to ensure adherence to infection prevention practices); perform hand hygiene prior to catheter insertion or manipulation; avoid using the femoral vein in obese adult patients for non-emergent insertion; use an all-inclusive catheter cart or kit; use ultrasound guidance for internal jugular catheter insertion; use maximum sterile barrier precautions during insertion; and use an alcoholic chlorhexidine antiseptic for skin preparation. Recommended practices for preventing infections after insertion include: disinfecting catheter access sites before accessing the catheter; removing nonessential catheters; changing transparent and gauze dressings and performing site care with a chlorhexidine-based antiseptic at specific intervals for non-tunneled CVCs; replacing administration sets (except those used for blood, blood products, or lipids) at intervals not longer than 96 hours; and performing surveillance for CLABSI [75].

Although the efficacy of the CLABSI prevention bundle has not been studied in HSCT recipients, all five elements of the bundle are recommended for this patient population. In addition, despite growing literature on antibacterial-impregnated catheters, the 2009 Guidelines do not require the use of such catheters [1]. However, antibacterial-impregnated catheters and other measures such as chlorhexidine-impregnated dressings, antiseptic-containing connector caps, and antimicrobial locks are special approaches that may be considered, after a risk assessment, for use in locations and/or populations within hospitals when CLABSIs are not controlled by use of basic practices [75].

Patients and caretakers should be trained in the care of intravascular devices (AIII) [77]. Catheter and access site contact with tap water should be avoided and patients should cover the catheter ends during bathing or showering with an impermeable product (BIII). To reduce the risk of inadvertent catheter contamination, intravenous infusions given outside the hospital should be started by a caregiver rather than the patient (BIII) [78].

Despite meticulous catheter care, infections may occur; these are often related to skin flora, particularly staphylococci. For catheter-related infections occurring before transplantation, catheter removal, along with the administration of an appropriate course of intravenous antibiotics, is preferred to attempts to sterilize and use the same catheter. For post-transplant infections, catheter removal is preferred in many instances, but may be impractical in the setting of severe

thrombocytopenia. CLABSIs with coagulase-negative staphylococci are often possible to clear with the catheter in place, so an attempt to treat through the same catheter may be made as long as no tunnel infection is present and the bacteremia clears promptly. If one is attempting to clear a catheter infection with the catheter in place, the catheter lumens should be used sequentially for antibiotic administration. Regardless of whether the catheter is removed, repeat blood cultures should be performed after the completion of the course of intravenous antibiotics to document eradication of the infection. If fever or bacteremia fails to clear promptly, septic thrombophlebitis or, less commonly, endocarditis may be present, and these should be sought with appropriate ultrasound testing and echocardiography. The presence of endovascular infectious complications lengthens the duration of antibiotic therapy and may alter the timing of transplantation.

45.5 Food and Nutrition

In the early days of bone marrow transplantation, sterile or very low-microbial diets were recommended as part of a total protective environment, often in conjunction with the use of selective gut decontamination with oral nonabsorbable antibiotics. In recent years, sterile diets have been much less commonly used. The 2009 Guidelines do not comment on dietary restrictions post-transplantation, but the earlier guidelines published in 2000 recommended a low-microbial diet (BIII) for 3 months after autologous HSCT transplantation and until discontinuation of immunosuppression in those undergoing allogeneic HSCT transplantation [2]. Several studies have found no difference in infection rates in HSCT recipients that consume diets that exclude raw fresh fruits and vegetables [79–81], calling into the question the necessity of adhering to a low-microbial diet post-transplantation.

HSCT recipients should not consume any raw or undercooked meat, eggs, or seafood, and they and their caregivers should follow standard recommendations for food preparation and cleaning of utensils and surfaces [2]. Avoiding foods associated with listeriosis, including soft cheeses, hot dogs, turkey franks, and deli meats, is prudent for HSCT recipients. If these individuals eat leftovers, they should be reheated to steaming hot. Fast-food restaurants should also be avoided, especially in the early months after transplantation. HSCT recipients should also be reminded that naturopathic medications may contain molds [2], especially if they are derived from plant substances.

Total parenteral nutrition (TPN) may be necessary on a temporary basis for patients with severe mucositis or gastrointestinal GVHD. The issues concerning TPN in HSCT recipients have been reviewed [82]. Several studies have suggested that the addition of glutamine may have a beneficial effect in decreasing hospital stay and the incidence of positive blood cultures [82, 83]. Whether administration of intravenous lipids affects patients' risk of infection remains unclear; several studies have identified TPN as a risk factor for CLABSI [75], whereas one study showed no difference in bacteremia and fungemia rates with a moderate dose of intravenous lipids versus a low dose [84].

With the recent legalization of marijuana for medical use in several states in the USA, use of marijuana for control of nausea, pain, and appetite stimulation will likely increase in the coming years. There have been several case reports in the literature of patients with hematopoietic malignancies developing invasive *Aspergillus* infections following regular marijuana use, as *Aspergillus* and other molds can contaminate natural preparations of the drug [85, 86]. Though there are as yet no formal guidelines addressing this issue, HSCT recipients and candidates should avoid marijuana, particularly in its inhaled form, to minimize risk of invasive mold infections.

45.6 Control of Specific Health Care-Associated and Community-Acquired Pathogens

45.6.1 Legionella Species

Legionellosis (or Legionnaire's disease, LD) should be considered in the differential diagnosis of HSCT recipients who develop pneumonia (AIII) [4, 46, 87]. The incubation period is 2–10 days. Thus, patients with laboratory-confirmed LD who are hospitalized for more than 10 days before symptom onset have definite health care-associated legionellosis, and those who are hospitalized between 2 and 9 days before symptoms may have health care-associated infection. If LD is confirmed in a patient hospitalized on the HSCT unit for all or part of the 2–10 days before illness onset or if two or more cases of proven LD occur in patients visiting an outpatient HSCT clinic, the case(s) should be reported to the local or state health department if the disease is reportable in that region (AIII) (the reporting requirements of health departments can vary by region) and a thorough environmental and epidemiologic investigation should be performed by the infection control team (AI) [4, 46, 87]. This should include an assessment of cooling towers, hot water tanks, showers, and tap water faucets. In the United Kingdom, one survey of the water supplies of 85% of transplant units revealed *Legionella* species in 55% [88].

HSCT centers should follow published recommendations regarding the prevention of nosocomial LD (BIII) [4, 46]. Only sterile water should be used to fill nebulization devices and to rinse respiratory-care equipment following cleaning or disinfection (BII) [46]. Use of large volume air humidifiers that create aerosols should be avoided (DI), unless these devices are sterilized or subjected to daily high-level disinfection with sterile water (CIII) [46]. When a new hospital with an HSCT center is built, the cooling tower should be placed such that it is directed away from the hospital's air intake system and aerosol production should be minimized (BII) [46].

Decorative fountains should not be installed in HSCT units or in areas frequented by HSCT recipients (BIII). A clonal outbreak of LD linked to a decorative fountain has been reported [89]. Since HSCT recipients are at higher risk for disease and death from *Legionella*, periodic routine culturing for these organisms in water samples from HSCT center's potable water supply should be considered (CIII) [4, 46, 90, 91]. However, the optimal methodology and cost-effectiveness of environmental surveillance measures for *Legionella* have not yet been determined. The goal should be to maintain the water system free of bacteria (AIII) [4, 46], and if *Legionella* species are detected, the water supply should be decontaminated (AII) [92]. In this situation, patients should not take showers; instead, they should receive sponge baths with *Legionella*-free water (BIII). In addition, water from faucets contaminated with *Legionella* species should not be used in the HSCT unit, in order to prevent the creation of infectious aerosols (DIII). Finally, HSCT recipients should use sterile water for drinking, brushing teeth, or flushing nasogastric tubes (BIII) to prevent acquisition of *Legionella* and other water-borne pathogens.

45.6.1.1 Methicillin-Resistant Staphylococci

HSCT centers should follow published infection control guidelines to prevent health care-associated transmission of methicillin-resistant *S. aureus* (MRSA) [3, 93–96], including hand hygiene with either alcohol-based hand rub or soap and water before and after all contact with patients or potentially contaminated equipment or environment (AII); applying Standard Precautions to all patients at every encounter; use of Contact Precautions for patients colonized or infected with MRSA (AII); and compliance with standard environmental cleaning with an effective disinfectant (BIII) [3, 5].

Currently there is insufficient evidence to support routine screening of all HSCT recipients for MRSA or use of topical or systemic antimicrobial therapy for eradication of asymptomatic MRSA colonization; these are active areas of research. If basic infection control practices fail to prevent high rates of MRSA infection, consideration should be given to implementation of the following adjunctive strategies: collection of MRSA surveillance cultures on admission and serially throughout the hospitalization (BII) [97], with or without decolonization therapy (BII) [98]; routine bathing with chlorhexidine (BIII) [99]; cohorting of MRSA patients or placement of all such patients in single rooms; and designation of dedicated staff to the care of MRSA patients (CIII) [96].

The optimal duration of Contact Precautions for patients is unknown, though studies have demonstrated that patients can remain colonized for extended periods of time. HSCT centers may use different discontinuation criteria for the removal of Contact Precautions, including continuation of such precautions until antimicrobial therapy for MRSA is complete and three consecutive screening cultures collected on separate days are negative (CIII) [95]. For patients with recurrent MRSA infections, eradication of organism carriage can be attempted using any and/or all of the following techniques: application of 2% mupirocin calcium ointment to the nares, chlorhexidine bathing, or administration of systemic antibiotics, though none of these strategies have been shown to be consistently effective (CII) [100, 101]. High-level mupirocin resistant MRSA isolates have emerged in Europe, South America, and the Middle East [102–104], though they remain uncommon in the USA. Therefore, mupirocin use should be coordinated with the hospital infection control team to prevent incorrect use or overuse of this product, which may lead to antimicrobial resistance.

45.6.1.2 Staphylococci with Reduced Susceptibility to Vancomycin

Fortunately, staphylococci with reduced susceptibility to vancomycin are rare [105]. All HSCT centers should have laboratory facilities capable of performing antimicrobial susceptibility testing on all staphylococci (AII) [106–108]. In addition, routine surveillance for staphylococci with reduced susceptibility to vancomycin, which is defined as a vancomycin minimal inhibitory concentration (MIC) of greater than or equal to 2 mcg/mL for *S. aureus* and of at least 4 mcg/mL for coagulase-negative staphylococci, should be conducted (AIII). If such isolates are identified and confirmed, infection control personnel should be notified immediately and institute published guidelines for control of such isolates (BII) [107, 109, 110]. Current recommendations suggest that prudent use of antibiotics by HSCT centers, especially vancomycin, is essential in preventing the emergence of resistant staphylococci (AII). Antimicrobial use patterns in the facility should be monitored to prevent overuse of antimicrobials, including vancomycin (AII) [107, 109, 110].

45.6.1.3 Vancomycin-Resistant Enterococcus (VRE)

VRE infection is associated with poor outcomes in HSCT recipients [111, 112]. To reduce the risk of VRE infection, care providers should limit the use and duration of treatment with vancomycin and anti-anaerobic agents (AII) [107, 113–115]. Although oral vancomycin promotes overgrowth of VRE in the bowel, the risk of VRE acquisition should not be a major consideration when selecting oral vancomycin for the treatment of severe or recurrent *C. difficile* infection (BIII) [115].

Patients colonized with VRE usually remain colonized for prolonged periods of time, which can extend beyond the index hospitalization [116, 117]. Furthermore, VRE can re-emerge after previous negative cultures when the patient is exposed to antimicrobials [95]. For these reasons, Contact Precautions for HSCT recipients with prior VRE colonization

or infection should be continued during subsequent hospital admissions (AIII), though some centers do have protocols for removing VRE designations after a specified number of negative surveillance cultures. The 1995 HICPAC Guidelines for preventing the transmission of VRE suggest three negative stool/perianal cultures obtained at weekly intervals as a criterion for discontinuation of Contact Precautions [118, 119]. Adherence to the same infection control practices described in the MRSA section above is recommended to prevent VRE transmission in HSCT units (AII), including: hand hygiene; applying Standard Precautions to all patients at every encounter; use of Contact Precautions for patients colonized or infected with VRE; and compliance with standard environmental cleaning with an effective disinfectant [3, 5]. Eradication of VRE carriage has not been adequately studied and should not be attempted (DIII). The efficacy of surveillance cultures for VRE to prevent health care transmission of this pathogen is unclear. If there is evidence of nosocomial transmission of VRE in a HSCT center, use of VRE rectal or stool surveillance cultures to identify colonized patients may be considered (CIII) [93].

45.6.1.4 Multidrug-Resistant Gram-Negative Bacilli (MDR-GNB)

MDR-GNB are defined as GNB that are resistant to one or more classes of antimicrobial agents, including those that produce extended-spectrum β-lactamases (ESBL) and carbapenemases. In 2006, HICPAC published a detailed discussion of MDR-GNB and recommendations for their prevention [95]. Thoughtful use of antimicrobial agents is essential to limit the development of MDR-GNB (BII). In addition, adherence to the same infection control practices described in the MRSA section above is recommended to prevent MDR-GNB transmission in HSCT units (AII), including: hand hygiene; applying Standard Precautions to all patients at every encounter; use of Contact Precautions for patients colonized or infected with MDR-GNB; and compliance with standard environmental cleaning with an effective disinfectant [3, 5]. Successful control of MDROs has been documented in the USA and abroad using a variety of combined interventions. These include improvements in hand hygiene, use of Contact Precautions until patients are culture-negative for a target MDRO, active surveillance cultures, education, enhanced environmental cleaning, and improvements in communication about patients with MDROs within and between healthcare facilities.

The use of multiple concurrent control measures in these reports underscores the need for a comprehensive approach for controlling MDROs [120]. Evidence is mixed regarding whether active surveillance programs for MDR-GNB are useful in addition to basic infection control practices. Several studies reported a decrease in ESBL-producing *Enterobacteriaceae* over a 6-year period using a multipronged approached that included surveillance cultures [121, 122]. Other reports suggest that routine surveillance cultures are not required to control nosocomial MDR-GNB transmission [123]. That said, the CDC now recommends that a single round of surveillance cultures be sent in high-risk settings (i.e., hospital settings in which patients are exposed to broad spectrum antibiotics) if previously unidentified carbapenemase-producing GNB are isolated [120]. HSCT units suffering from high rates of MDR-GNB infection should implement and/or ensure basic infection control measures are in place. The use of active surveillance cultures as an adjunct to their standard infection control practices may also be considered (CIII).

45.6.1.5 Clostridium Difficile-Associated Diarrhea (CDAD)

In the mid-2000s, an epidemic strain of *C. difficile* spread rapidly in hospitals. HSCT centers should follow published recommendations for prevention, control and treatment of CDAD (AI) [7, 124, 125]. The 2014 Updated Guidelines by SHEA, IDSA, the American Hospital Association (AHA), and the Association for Professionals in Infection Control and Epidemiology (APIC) emphasize the importance of limiting inappropriate antimicrobial use in order to prevent CDAD in the hospital [7]. Patients with CDAD should be placed on Contact Precautions for the duration of their illness (BIII) [3, 7, 125], and placed in a private room, if possible [7]. HCWs entering the patient's room should wear gowns (BIII) and gloves (AI) regardless of whether they anticipate touching the patient or anything in the environment [3, 7, 124, 125]. The 2014 SHEA/IDSA/AHA/APIC Guidelines recommend placing patients with diarrhea on Contact Precautions while *C. difficile* testing is pending to limit transmission at a time when bacterial shedding is greatest [7]. Contact Precautions should remain in place at least until the patient is asymptomatic (BIII) [124], or at least 48 hours thereafter [7]. A "test of cure" to determine whether Contact Precautions can be discontinued is discouraged (DII) [7, 124]. If there is evidence of ongoing transmission of *C. difficile* despite compliance with basic prevention strategies, HSCT centers should consider maintaining Contact Precautions until hospital discharge, even if diarrhea resolves sooner (CIII) [7]. The following practices are **not** recommended for prevention of nosocomial *C. difficile* transmission: routine stool surveillance cultures or toxin assays for *C. difficile* among asymptomatic patients or HCWs (DIII); culture of *C. difficile* from hand swabs of HCWs (EIII); and treatment of asymptomatic *C. difficile* carriers (EII) [7]. Furthermore, the prophylactic use of lyophilized *Saccharomyces boulardii* to prevent diarrhea among HSCT recipients receiving antibiotics is contraindicated, as it has been associated with development of *S. boulardii* fungemia [126].

CDAD is one of the situations where hand washing with antimicrobial soap and water is preferred over alcohol-based hand rubs, as the latter are not sporicidal for *C. difficile.*

Proper hand washing technique, including washing for a minimum of 15–30 seconds, should be enforced (BI) [5]. *C. difficile* is more resistant than many organisms to standard disinfectants. An intervention study suggested that the use of 1 to 10 hypochlorite solution was more effective as an environmental disinfectant than was quaternary ammonium in the control of *C. difficile* in HSCT recipients [127]. The incidence of *C. difficile* diarrhea decreased from 8.6 to 3.3 cases per 1000 patient-days when the disinfectant was changed to hypochlorite and then increased again to 8.1 cases per 1000 patient-days when quaternary ammonium was again used [127]. The use of bleach-containing cleaning products for environmental disinfection should be considered if there is ongoing evidence of *C. difficile* transmission with standard procedures (BII). A variety of products with EPA-registered claim to activity against *C. difficile* are becoming available, including those that do not contain bleach. The optimal frequency and extent of cleaning with such agents remain uncertain, though at a minimum cleaning should be performed daily and when surfaces are visibly soiled; the agents should be applied for contact times recommended by the manufacturer. The 2008 Guideline for Disinfection and Sterilization in Healthcare Facilities gives further recommendations regarding environmental cleaning, and disinfection of equipment such as colonoscopes, to prevent transmission of *C. difficile* [6].

45.6.1.6 Community-Acquired Respiratory Virus Infections

CRVs can cause significant morbidity and mortality in HSCT recipients, especially those in the preengraftment phase [128, 129], and may produce health care-associated outbreaks if they are introduced into an HSCT center. Influenza, parainfluenza, adenovirus, respiratory syncytial virus (RSV), and human metapneumovirus (hMP) can produce severe disease, although reported mortality rates have varied. Pulmonary copathogens are common, possibly accounting for some of the observed mortality in CRV-infected patients [130].

Measures to prevent the introduction and spread of CRVs on the HSCT unit should be implemented (AIII) [46, 131]. To prevent health care-associated transmission, identifying HSCT recipients with respiratory virus symptoms and placing them under appropriate precautions is crucial. Patients should empirically be placed on Contact plus Droplet Precautions until a specific pathogen has been identified, after which these precautions can be modified based on the results (BIII). Droplet Precautions alone are appropriate for influenza, but RSV, parainfluenza, and adenovirus require both Contact and Droplet Precautions. Primary or disseminated varicella infection requires Airborne plus Contact Precautions, though single dermatomal zoster only requires Standard Precautions [3]. When a patient at the HSCT center has a viral upper or lower respiratory tract infection, HCWs and visitors should follow all Standard and Transmission-Based Precautions, with attention to hand hygiene before and after contact with a patient, after handling respiratory secretions or objects contaminated with respiratory secretions, and before donning and after doffing gloves (AII) [5, 46]. This practice is important because transmission of most CRVs occurs by contact, especially from hand to nose and eye. In keeping with Standard Precautions, HCWs should wear a face mask that fully covers the front and side of the face while performing aerosol-generating procedures—such as bronchoscopy, endotracheal intubation, and open suctioning of the respiratory tract—to avoid contamination with respiratory secretions (AIII). For patients with suspected or known influenza, such procedures should be performed using Airborne Precautions [132]. In outpatient waiting rooms, patients with CRV infections should be separated from other patients and should be encouraged to use respiratory hygiene/cough etiquette (BIII). Patients and their companions should be screened upon arrival for symptoms of CRV infections; those screening positive should be educated about proper use of facemasks, tissues, and hand hygiene, and should be provided with the supplies to implement these practices [132].

To minimize the risk of CRV spread on the HSCT unit, HCWs and visitors should be screened for upper respiratory infection symptoms (BII), and if present, should be restricted from contact with HSCT recipients and from individuals undergoing conditioning therapy (BIII) [51, 52]. HCWs should be re-assigned to non-patient care duties (BIII) and visitors should refrain from visiting (AII) until symptoms resolve. For influenza in particular, HCWs should be excluded from work for 7 days following symptom onset (BIII) [132]. HCWs and visitors with infectious conjunctivitis should be restricted from direct patient contact until drainage resolves (AII) [51].

All HSCT recipients or candidates with signs and symptoms of possible CRV infection should be promptly tested for the presence of such viruses (BIII). Nasopharyngeal throat swabs, washes, or aspirates and bronchoalveolar lavage fluid should be tested by viral culture, rapid antigen testing, or PCR (BIII). This strategy facilitates timely initiation of Transmission-Based Precautions and, in some instances, preemptive treatment of certain CRVs to prevent disease complications [46]. Retesting of patients may be performed to help determine whether patients have stopped shedding viruses following resolution of their symptoms (BIII). Prolonged CRV shedding may occur in HSCT recipients; viral shedding has been reported to last more than 22 days for RSV, 4 months for influenza, and 2 years for adenovirus [133–136]. Appropriate isolation precautions should be maintained for at least the duration of illness (AII), and consideration should be given to continuing them for the duration of hospitalization or viral shedding to prevent nosocomial transmission (CIII).

Routine virologic screening of asymptomatic HSCT candidates is performed by some HSCT centers to detect outbreaks and implement infection control measures early, though there is insufficient data at present to recommend this

practice. During outbreaks of RSV in a HSCT unit, HCWs may be cohorted to minimize nosocomial transmission [54, 137], though the 2009 Guidelines do not recommend this strategy for control of other CRV outbreaks. Two winter community RSV outbreaks were controlled using the following strategies on an adult bone marrow transplantation unit: identification, isolation, and cohorting of RSV patients; use of masks and gloves; prompt therapy with aerosolized ribavirin; screening of visitors; restricting visitation by children younger than 12 years of age and anyone with RSV symptoms; and restricting symptomatic hospital staff from working in the bone marrow transplantation unit [137]. Nasopharyngeal antigen testing of symptomatic individuals, Contact Precautions, and infection control education for HCWs controlled a parainfluenza virus outbreak on a bone marrow transplantation unit [138]. Weinstock and colleagues reported an outbreak of influenza A on a 30-bed HSCT unit and described interventions implemented to terminate the outbreak and to prevent nosocomial influenza the following season [139]. These interventions included strict isolation of patients with influenza, reverse isolation of all other patients on the unit during the outbreak, more rapid laboratory turnaround of diagnostic tests for influenza, restriction of visitors, cohorting of HCWs assigned to the unit, universal antiviral prophylaxis of all patients and HCWs on the unit during the outbreak, and an enhanced influenza vaccine campaign for HCWs the following season [139].

HCWs and close contacts of HSCT recipients should be vaccinated yearly for influenza, using the inactivated vaccine rather than the live attenuated vaccine to eliminate the risk of vaccine virus transmission (AI) [56]. Under certain conditions involving CRV outbreaks, influenza vaccine along with prophylactic antiviral treatment of a subgroup of patients, an entire unit, and/or family members and HCWs may be undertaken, particularly in the case of an influenza outbreak that is not controlled with other measures or that involves an influenza strain that is not contained in the current vaccine (BIII) [1, 54, 139]. Some clinicians have recommended chemoprophylaxis for all HSCT recipients who are less than 24 months from transplantation, as well as for those beyond 24 months who are still receiving immunosuppressive therapy [2]. However, ongoing changes in antiviral resistance require that any prophylaxis decisions be based on updated CDC recommendations regarding the strains and outbreaks that are occurring during a particular season [140].

45.6.1.7 Adenovirus

Adenovirus can cause large outbreaks in acute and chronic care facilities, including outbreaks of highly symptomatic respiratory and gastrointestinal infection [141]. Transmission can occur in multiple ways, including inhalation of aerosolized droplets, contact with contaminated surfaces, fecal–oral spread, exposure to infected tissue or blood, and rarely via contaminated water [142]. Strict infection control measures are required to prevent spread of this easily transmissible virus. Sputum and oral secretions of infected adults contain 10^6–10^7 viral particles/ml and the virus can survive up to 35 days on surfaces. As few as five virions are required to cause infection in immunocompromised adults [143], who can then asymptomatically shed the virus for months following infection. Patients with adenovirus gastroenteritis should be placed on Contact Precautions, while patients with respiratory illnesses, conjunctivitis, or disseminated disease require both Droplet and Contact Precautions to prevent nosocomial transmission [3]. Hand hygiene with either an alcohol-based rub or soap and water and standard environmental disinfectants effectively kill the virus [144, 145].

45.6.1.8 Viral Gastroenteritis

Like CRV, viral gastroenteritis can cause more severe disease in HSCT recipients than in normal hosts, and health care-associated outbreaks can develop if these pathogens are introduced into a HSCT unit. Commonly encountered pathogens include adenovirus (see above), norovirus, rotavirus, and astroviruses; all are spread via the fecal–oral route, and contact with infected persons and contaminated fomites. Compliance with hand hygiene, appropriate isolation precautions, and environmental cleaning are required to prevent nosocomial transmission of these pathogens (AII) [4]. At a minimum, isolation precautions should be maintained for the duration of the illness (AII), with consideration given to continuing these measures for the duration of the hospitalization or the duration of viral shedding, since HSCT recipients may asymptomatically shed virus following symptom resolution (CIII).

Rotavirus is the most common cause of severe gastroenteritis in the pediatric population worldwide. Due to high concentrations of viral shedding in stool and the ability of the virus to persist in the environment for more than 10 days, environmental contamination is common [146, 147]. Health care-associated outbreaks of rotavirus have been traced to contaminated toys and hands [47, 148]. Contact Precautions are required to prevent transmission of rotavirus (AIII). Alcohol-based hand rubs effectively kill the virus and are sufficient for hand hygiene, unless hands are visibly soiled. Prompt removal of soiled diapers and thorough environmental cleaning are essential to limit environmental contamination with rotavirus (AIII) [3].

Norovirus is the most common cause of outbreaks of nonbacterial gastroenteritis, and now causes >50% of foodborne infections in the USA. This virus is easily transmissible and less than 100 particles are required to cause infection in normal hosts. Immunocompetent individuals continue to shed virus up to 72 hours following symptom recovery, whereas some immunocompromised hosts can shed virus for months to years following infection. Unlike rotavirus, alcohol-based hand rubs and standard disinfectant agents do not effectively kill norovirus [3, 149]. Multiple infection control measures

should be implemented to prevent transmission, including: hand washing with soap and water, Contact Precautions, wearing masks while cleaning areas contaminated by feces and vomitus, and minimal handling of soiled bedding and garments (BIII) [1, 8]. Per the 2011 HICPAC Guidelines for the Prevention and Control of Norovirus Gastroenteritis Outbreaks in Healthcare Settings, Contact Precautions should be maintained for at least 48 hours after symptom resolution, and consideration should be given to extension of these precautions and to cohorting infected individuals if they have medically complex conditions, such as HSCT recipients and candidates [8]. A hypochlorite-based cleaning agent should be used on non-porous environmental surfaces (BII) [150]. Environmental cleaning and disinfection should be performed at least daily, when items are visibly soiled, and following hospital discharge (BIII). Heat disinfection to 60 °C can be utilized for items that cannot be cleaned with bleach-based disinfectants [151]. HCWs with norovirus should be excluded from work at least 48 hours after symptom resolution [8]. In addition, staff cohorting protocols should be implemented in the event of a norovirus gastroenteritis outbreak [8].

Astroviruses have caused outbreaks of viral gastroenteritis in hospitals, nursing homes, and daycare centers. The virus can survive in the environment for several months [4]. Contact Precautions and environmental disinfection are effective and should be implemented at HSCT centers during outbreaks (AII).

45.6.1.9 Tuberculosis

Tuberculosis (TB) may reactivate in HSCT recipients in the face of immunosuppression. Though the 2009 Guidelines do not comment on TB prevention methods, the 2000 Guidelines recommended that HSCT candidates be screened by history and chart review for a history of exposure to tuberculosis (AIII). A tuberculin skin test (TST) using five tuberculin units of either the Tubersol (Connaught-Aventis, Swiftwater, PA) or Aplisol (Monarch, Bristol, TN) formulations may be administered by the Mantoux method. This test result may be unreliable, however, because of the patient's baseline immunosuppression. For immunocompromised hosts, a positive skin test is defined as 5 mm or more of induration [152]. HSCT candidates with a recently positive skin test or a previously positive skin test and no prior preventive therapy should have a chest radiograph and an evaluation for active tuberculosis (AI). Studies are mixed regarding the efficacy of the interferon-γ-release assays (IGRA) in HSCT recipients. One study suggested that IGRA is a more sensitive and specific test than the TST for predicting active tuberculosis after transplantation in HSCT recipients [153], while a second found no difference in rates of positive IGRAs and TSTs and poor agreement between these test outcomes regardless of previous BCG vaccination [154].

HSCT centers should adhere to published recommendations concerning the control of TB in health care facilities. These guidelines include the institution of Airborne Precautions and use of negative-pressure rooms for patients with suspected or confirmed laryngeal or pulmonary tuberculosis (AII) [3]. When HCWs are caring for such patients, they should wear N95 respirators or Powered Air Purifying Respirators (PAPRs) during exposure to these potentially infected patients, including in airborne infection isolation rooms and when HEPA filters are deployed (AIII). To obtain a maximal benefit in their use, HCWs should be fit-tested and trained in the use of N95 respirators and/or PAPRs annually (AIII).

Vaccination with the Bacille Calmette-Guérin (BCG) vaccine is contraindicated in HSCT candidates and recipients because of the potential for disseminated and fatal infection due to BCG in immunocompromised hosts (EII) [155].

45.7 Infection Control Surveillance

HSCT centers should follow standard guidelines for surveillance of epidemiologically significant nosocomial pathogens and their susceptibility patterns, including MRSA, VRE, MDR-GNB, *C. difficile*, CRVs, and invasive mold infections (BIII) [3]. In the absence of clusters of infections, surveillance cultures of the environment or of equipment or devices used for pulmonary function testing, the delivery of inhalation anesthesia, or respiratory care are not indicated (DIII) [46]. In the absence of an outbreak of nosocomial fungal infection, HSCT centers should not perform routine fungal surveillance cultures of devices or dust in patient rooms (DIII). Routine sampling of air, ceiling tiles, ventilation ducts, and filters for mold detection is classified as CIII, and thus it is optional [1]. However, infection control surveillance for clinical cases of aspergillosis in HSCT recipients is advisable (BIII). An increase in the number of cases should trigger an investigation for potential environmental sources of mold exposure in the HSCT center (BIII). The ventilation system should also be evaluated to ensure appropriate filtration, air flow, and air pressure differentials (BIII) [9].

45.8 Summary

In the decades since the performance of the first bone marrow transplants, considerable experience has been gained. Advances in many areas have altered the topography of infection risk for the HSCT recipient. These include reduced intensity conditioning, use of growth factors, prophylaxis and preemptive therapy for infections, identification of emerging pathogens and techniques to facilitate their early diagnosis, availability of new and effective antimicrobials, and strategies for prevention and treatment of GVHD. Nevertheless, although agreement exists on general principles, basic issues such as optimal infection control measures remain unresolved to some extent. The 2009 Guidelines provide a comprehensive review

of literature up to that date. However, the relative paucity of recommendations for levels AI, AII, BI, and E in the infection control guidelines is striking. Among the AI and AII recommendations, most are global and generic recommendations that are derived from, and applicable to, other clinical settings. Therefore, there is a need for more evidence-based studies to define optimal infection control practices in these high risk patients undergoing HSCT. For future developments, the reader is advised to consult the CDC website (www.cdc.gov) for updated recommendations on infection control, prophylaxis, outbreaks, and emerging pathogens which may go beyond the recommendations cited in this chapter.

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Infection Prevention and Control Issues After Solid Organ Transplantation

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Transplantation has been accepted as a treatment modality for terminal organ failure. Therapies used to prevent rejection suppress the immune system and as a result, the transplant recipient is often at high risk of infection. Prolonged and frequent exposure to healthcare settings and multiple antibiotics may predispose the transplant recipient to colonization or infection with multidrug-resistant organisms. The use of good infection prevention and control practices is extremely important throughout the continuum of care for solid organ transplant (SOT) recipients. In the hospital setting, antimicrobial-resistant pathogens often cause the infections identified during admission or after discharge, resulting in increased morbidity and mortality.

This chapter reviews selected infection prevention and control practices that address common infections in transplant recipients. The U.S. Centers for Disease Control and Prevention (CDC) has issued guidelines for the prevention of infection for hematopoietic stem cell transplantation (HSCT) recipients, but not specifically for SOT recipients. Pertinent guidelines on infection prevention and control issues have been developed by the CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC) to provide specific recommendations that are pertinent to all patient populations. Guidelines referenced in this chapter include the Guideline for Hand Hygiene in Health-Care Settings—2002 [1], Guidelines for Preventing HealthCare-associated Pneumonia, 2003 [2], Guidelines for Environmental Infection Control in Health-Care Facilities, 2003 [3], Management of Multidrug-Resistant Organisms in Healthcare Settings, 2006 [4], Guidelines for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings, 2007 [5], and the Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008 [6]. The American Transplant Society has published guidelines for the management and prevention of infections in organ transplant candidates and recipients which address specific aspects of infection control practices pertinent to transplantation [7]. The Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA) have also published a compendium of strategies to prevent healthcare-associated infections (HAIs) in acute care hospitals [8]. These strategies, most recently revised in 2014, highlight basic prevention practices that are often referred to as "bundles," guidance to infection control programs regarding implementation of these practices, as well as special approaches for infections that are not controlled using basic infection control practices. Despite the publication of expert guidance documents and guidelines some issues are still unresolved. The authors of this chapter describe some of the practices in their institutions, while acknowledging that different approaches to the same problem might exist.

46.1 Healthcare-Associated Infections

46.1.1 Prevention and Isolation Practices

Caregivers must maintain good infection prevention practices to minimize the transmission of infection in the healthcare setting. Invasive devices such as central venous catheters (CVCs), indwelling urinary catheters, and ventilators expose the patient to additional risks for infection. Most facilities have implemented infection prevention "bundles" designed to prevent these device-associated HAIs. Due to the success seen in reducing HAIs, the Centers for Medicare and Medicaid Services (CMS) issued new guidelines. After October 1, 2008, hospitals no longer receive additional payment for cases in which selected conditions were not present on admission, which include CVC-associated bloodstream infections and catheter-associated urinary tract infections [9]. What this means to hospitals is that claims are paid as though the secondary diagnosis was not present. These "Hospital-Acquired Conditions" (HACs) are considered "never events," but may still be problematic in transplant recipients. Careful attention must be given to good hand hygiene practices and CVC care, as well as to practices that decrease the risk of catheter-associated urinary tract infection. As a protective

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measure, patients may also be placed into protective precautions to heighten the awareness of the caregivers to the potential for serious infection.

Whereas the 1991 Occupational Safety and Health Administration (OSHA) Bloodborne Pathogens Standard focused primarily on employee protection [10], the CDC and HICPAC have published numerous patient-focused guidelines and recommendations for the prevention of HAIs. Revised in 2007, the Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings 2007 [5] updated and expanded the *1996 Guideline for Isolation Precautions in Hospitals*. The transition of healthcare delivery from primarily acute care hospitals to other healthcare settings (e.g., home care, ambulatory care, freestanding specialty care sites, and long-term care) created a need for recommendations that could be applied in all healthcare settings using common principles of infection control practice, but could be modified to reflect setting-specific needs. In this revision, the term "nosocomial infections" was replaced by "healthcare-associated infections" to better reflect the changing patterns in healthcare delivery. It may be difficult to determine the exact site of exposure to an infectious agent and/or acquisition of infection as patients move through the healthcare delivery system. The SARS experience, and more recently, the experience with Ebola virus disease, highlighted the need to better prepare for new emerging pathogens and focused on the ways minor breaks in infection control technique resulted in infections being transmitted to healthcare professionals. Sections of this guideline were created as evidence mounted that environmental controls could decrease the risk of fungal infections in severely immunocompromised patients. While the Protective Environment (PE) has been found to be of greatest benefit for patients undergoing allogeneic hematopoietic stem cell transplants, there may be some lessons to be learned from successful implementation in this group of patients. Organizational characteristics (e.g., nurse staffing levels and composition, establishment of a safety culture) are also identified as key components to promote adherence to recommended infection control practices. Combining the universal precautions and body substance isolation precautions, contact with human blood, body fluids, secretions or excretions (except for sweat), nonintact skin, mucous membranes, and contaminated items requires the use of personal protective equipment, as part of standard precautions. Respiratory hygiene/cough etiquette, safe injection practices, and the use of masks during insertion of catheters or injection of material into spinal or epidural spaces via lumbar puncture procedures were added to standard precautions in 2007. Transmission-based precautions are used, in addition to standard precautions, to prevent infections spread by airborne, droplet, and direct contact routes. Certain infections that had required disease-specific isolation precautions are now included under standard precautions.

Airborne precautions are used if a patient has a known or suspected infection with an agent that can be transmitted by evaporated droplets [droplet nuclei of <5 mm (micron)] that remain suspended in the air and that may be carried away from the infected patient. Measles, varicella, and tuberculosis are the primary infections included in this category; a patient infected with any of these must be housed in a room with controlled ventilation. Specialized air filters and negative pressure in the room prevent the infectious droplet nuclei from entering the general air supply and infecting others.

Certain diseases, such as influenza and adenovirus, generate droplets larger than 5 mm. These larger-sized particles are too big to remain suspended in the air; therefore, no special ventilation is required. Close contact with respiratory tract secretions is required for disease transmission, so masks should be worn by healthcare workers when they are working within 3 ft (0.9 m) of an infected patient to prevent the inhalation of infectious droplets.

Contact precautions are used to prevent the transmission of certain microorganisms that may be found on the patient's skin or on inanimate objects in the patient's environment. Included in this category are epidemiologically significant organisms, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), *Clostridium difficile*, and respiratory syncytial virus (RSV). Private rooms are recommended, but patients colonized or infected with the same organism(s) may be cohorted together, if necessary. If neither of these options is achievable, the immunosuppressive state of potential roommates should be evaluated. For example, placing a VRE-colonized patient with an otherwise healthy 30-year-old who has a broken leg would be preferable to placing that patient with a postoperative transplant recipient who might become more easily colonized and infected. Contact precautions require gloves and gowns be worn for contact with the patient or potentially contaminated items and areas in the patient room. While the likelihood of transmission to other patients through contact with clothing is remote, caregivers are likely to touch their own clothing (e.g., lab coat pockets) and thus transmit the organism on their hands. The 2006 HICPAC/CDC guideline, The Management of Multidrug-Resistant Organisms in Healthcare Settings, recommends donning gowns and gloves upon room entry and discarding them before exiting the room of a patient with organisms that have been implicated in environmental transmission (e.g., VRE, *C. difficile*, noroviruses and other intestinal tract agents, and RSV) [4].

The 1975 CDC isolation techniques manual defined a protective isolation category to protect neutropenic or immunosuppressed patients. Whereas other isolation categories were designed to prevent the transmission of disease from an infected patient to others, the purpose of protective or "reverse" isolation is to protect the highly susceptible patient. Neutropenic precautions are practices designed to reduce microbial contamination in the patient's environment. Because many infections in immunosuppressed patients are attributable to the patient's endogenous flora, the use of special environmental precautions is not recommended, except for allogeneic stem cell transplant recipients, for whom a

TABLE 46-1. Centers for disease control and prevention isolation precautions for selected infections

Infection	Precautions	Comments
Abscess, draining (minor)	Standard	Contact for major draining abscess
Adenovirus	Droplet, contact	
Aspergillosis	Standard	
Candidiasis	Standard	
Cellulitis	Standard	
Clostridium difficile	Contact	Private room preferred
Cytomegalovirus	Standard	
Epstein–Barr virus	Standard	
Fungus, endemic	Standard	Blastomycosis, coccidioidomycosis, histoplasmosis
Hepatitis, viral (HBV and HCV)	Standard	
Herpes simplex virus	Standard Contact	Encephalitis, recurrent mucocutaneous—skin/oral/genital Disseminated or severe mucocutaneous
Influenza	Droplet	
Legionnaires' disease	Standard	
Listeriosis	Standard	
Multidrug-resistant organisms (MRSA, VRE, and MDR-GNB)	Contact	Gown and gloves recommended on entry into room Private room preferred
Mycobacteria, nontuberculous	Standard	
Nocardiosis	Standard	
Parainfluenza	Contact	Respiratory infection in infants and young children
Parvovirus B19	Droplet	
RSV	Contact	
Rotavirus	Contact	
Tuberculosis	Airborne Airborne, contact	Pulmonary and/or laryngeal Extrapulmonary, draining lesion
Varicella-Zoster virus	Airborne, contact	Varicella (chickenpox), disseminated herpes zoster (shingles)
Zygomycosis (Mucor and Rhizopus)	Standard	

Abbreviations: *HBV* hepatitis B virus, *HCV* hepatitis C virus, *MRSA* methicillin-resistant *Staphylococcus aureus*, *MDR-GNB* multidrug-resistant gram-negative bacteria, *RSV* respiratory syncytial virus, *VRE* vancomycin-resistant enterococcus.

protective environment is necessary to minimize fungal spore counts in the environment and decrease the risk of invasive fungal infections [5]. To reduce the risk of infection, nursing care often focuses on skin integrity, indwelling intravenous devices, and good oral hygiene.

Isolation precautions for select organisms or disease syndromes are presented in Table 46-1. A complete list can be found in Appendix A of the CDC guideline on isolation precautions [5].

46.1.2 Definition of Healthcare-Associated Infections

The National Nosocomial Infections Surveillance (NNIS) system, a cooperative effort of the CDC and participating hospitals, began in 1970 with the purpose of creating a database to track nosocomial infections in the USA. In 2005, the National Healthcare Safety Network (NHSN) was established to integrate and supersede three surveillance systems at the CDC: the NNIS, the Dialysis Safety Network (DSN), and the National Surveillance of Healthcare Workers (NaSH). Patient-specific event data are entered into this Web-based system by individual facilities but comparative results can be found on the NHSN Web site (http://www.cdc.gov/nhsn) and are published annually in the *American Journal of Infection Control*. Many states have mandated that HAIs be reported through NHSN to better evaluate the magnitude of HAIs. The public reporting of infection data is state specific, ranging from all infections being reported in Pennsylvania to more limited requirements, such as primary bloodstream infections only. CMS requires reporting of certain HAIs as part of pay for performance initiatives. Healthcare facilities use the standardized definitions created by the CDC, previously in the NNIS program and now in the NHSN [11], to classify HAIs, thereby enabling comparisons to national benchmarks. For device-associated infections, such as ventilator-associated pneumonia, central line-associated bloodstream infection (CLABSI), and catheter-associated urinary tract infections, rates should be calculated (the denominator is device-days and numerator is the number of infections recorded; result is multiplied by a factor of 1000). Rates are thus recorded as the number of infections per 1000 device-days. HAI data are stratified into types of patient care areas (e.g., medical ICU, medical/surgical ICU, and SOT specialty care area) to provide infection rates according to the risk factors of the patient population served [12]. Operative procedure codes available for identification of surgical site infection (SSI) rates include liver, kidney, and heart transplant surgeries. Healthcare facilities reporting

HAIs to NHSN can get a standardized infection ratio (SIR) which is calculated by dividing the number of observed infections by the number of predicted (i.e., expected) infections. The number of predicted infections is calculated using infection probabilities estimated from multivariate logistic regression models constructed from NHSN data during a baseline period, which represents a standard population's infection experience. NHSN provides a p value and 95% confidence intervals to determine the statistical significance of the SIR for the healthcare facility's HAIs.

46.1.3 Central Line-Associated Bloodstream Infections

A general increase has occurred in the incidence of bloodstream infections caused by gram-positive bacteria, particularly *Staphylococcus* species. Many infections with coagulase-negative staphylococci are related to the increased use of various indwelling central lines. CLABSI is one of the primary infections seen in the immunosuppressed patient, because normal skin flora may colonize long-term access devices. In 2011, the CDC published Guidelines for the Prevention of Intravascular Catheter-Related Infections to help address this issue [13]. The incidence of infection varies with the type and intended use of intravascular devices. The two main device types are short term or temporary devices and those that are used for long-term access. Every device has some advantages and some risks. Attention should be focused on preventing site infections and on providing education for the patients and their caregivers if the catheter is not removed before hospital discharge.

The basic approaches to preventing CLABSI include the following [13]:

Before insertion

1. Educate healthcare personnel involved in the insertion, care, and maintenance of CVCs on CLABSI prevention.
2. Bathe ICU patients over 2 months of age with a chlorhexidine preparation on a daily basis.

At insertion

3. Use a catheter checklist to ensure adherence to insertion practices.
4. Perform hand hygiene before insertion or manipulation of a CVC.
5. Avoid the femoral vein for CVC access in adults, if possible.
6. Use maximal sterile barrier precautions during CVC insertion.
7. Use a chlorhexidine-based antiseptic for skin preparation in patients older than 2 months.
8. Use ultrasound guidance for internal jugular catheter insertion.

After insertion

9. Daily assessment of the need for the CVC and prompt removal of unnecessary CVCs.
10. Disinfect CVC hubs, connectors, and injection ports before accessing the CVC.
11. Change transparent dressings and perform catheter care with a chlorhexidine-based antiseptic every 5–7 days or immediately if the dressing is soiled, loose, or damp.
12. Use antimicrobial ointments for hemodialysis catheter insertion sites.

Several varieties of catheters and cuffs coated or impregnated with antimicrobial or antiseptic agents that reduce the risk of catheter-related bacteremia are available and have been shown to decrease the risk of CLABSI. Although catheters with chlorhexidine/silver sulfadiazine coatings, catheters impregnated with minocycline/rifampin or platinum/silver, and silver-coated cuffs may provide additional protection from skin flora [14, 15], they are more costly than the standard catheters and are recommended only if CLABSI rates could not be controlled using the basic approaches. The use of antimicrobial ointments for catheter insertion sites is no longer recommended, except for hemodialysis catheter insertion sites. Chlorhexidine-impregnated dressings may be beneficial in preventing CLABSI [16]. Daily chlorhexidine bathing may also reduce the rate of CLABSI in intensive care and bone marrow transplantation units [17, 18]. Although the routine replacement of catheters is unnecessary, the CVC site should be diligently monitored for evidence of infection. Guidewires should not be used for catheter exchange if any local redness, tenderness, or purulent material is present at the insertion site.

46.1.4 Prevention of Exposure from Healthcare Workers and Visitors

Employees and visitors may also transmit infections to the transplant recipient. Healthcare workers should undergo an evaluation of their health history and immunization status at the beginning of their employment [19]. Vaccination of healthcare workers who have no history of varicella infection or who are seronegative is strongly encouraged, because varicella can be life threatening in the SOT recipient. All staff members should receive the influenza vaccine annually, and, if they have not already been immunized for hepatitis B, they should receive hepatitis B vaccine at employment. Healthcare facilities should have well-defined policies to establish when potentially infectious personnel should not have patient contact. Employees should be encouraged to report any potential exposures or illnesses; human resource policies should permit temporary reassignment or furlough from duty to minimize the potential exposure of transplant recipients to

communicable infections. During the pretransplantation screening process, family members should be educated about infection prevention strategies and receive an annual influenza vaccination and ensure that their other vaccinations are up to date in order to better protect the transplant recipient [20]. Clinical personnel should monitor visitors for illnesses, such as colds, to prevent transmission. Posters may be displayed during flu season as additional reminders.

46.1.5 Fungal Infections

Despite the establishment of definition criteria, determining whether pneumonia is acquired in the hospital setting is one of the most difficult infections for the infection preventionist to classify. The CDC has three specific definitions of nosocomial pneumonia [11]. No defined incubation period exists when fungal pneumonia is suspected, so the traditional "onset of infection 48 hours after admission" standard that separates community-acquired infections from hospital-acquired infections is not valid. The isolation of fungal species from expectorated sputum may not be diagnostic, but clinicians often start antifungal therapy when they are encountered. These isolates could also represent transient colonization or a laboratory contaminant, not necessarily invasive disease [21]. Therefore, comparing fungal pneumonia rates among hospitals is difficult. Because of the ubiquitous nature of circulating fungal spores and of generally higher spore counts outdoors, determining whether a discharged patient who is readmitted with invasive fungal pneumonia acquired the infection while he or she was in the hospital is challenging. Comparative data on the incidence of nosocomial fungal pneumonia are unavailable, and many institutions have attempted to develop their own definitions of hospital-associated fungal pneumonia. Table 46-2 details the case definitions at some of the authors' institutions; an arbitrary hospitalization of 7 days prior to onset of infection is used to distinguish between hospital- and community-acquired fungal infections. A recent review of construction and renovation-related healthcare-associated fungal infections showed a decrease in number of outbreaks between 2010 and 2014, which may be due to effectiveness of infection prevention measures, or because of the high number of previously reported outbreaks [22].

Table 46-2. Case definitions

Term	Definition
Hospital-associated (nosocomial) infection	The patient has one or more positive cultures with the same pathogenic fungal species and clinical signs of infection and histopathologic or radiographic evidence of invasive fungal disease. OR Histopathologic or radiographic evidence of invasive disease with no microbiologic culture confirmation may be considered an infection if the patient is treated with an antifungal agent. Date of onset should be more than 7 days after admission with no evidence of active or incubating infection at the time of admission.
Colonization	Significant isolate(s) that cannot be classified as disseminated or locally invasive or if no systemic antifungal therapy is given.
Not significant	One isolate of a fungal species from a nonsterile site, no systemic antifungal therapy, or no correlation of routine microbiologic and fungal cultures.
Community-acquired infection	Signs or symptoms of infection are present at the time of admission and the patient was not hospitalized within the prior 2 weeks.

46.1.6 Aspergillosis

46.1.6.1 Environmental Concerns

Healthcare-associated aspergillosis is associated with the following three main mechanisms: airborne acquisition, which is typically secondary to contaminated ventilation systems; direct contact, through contaminated objects such as wound dressings; and airborne and contact, in which both mechanisms may be implicated, as is seen in sternal fungal osteomyelitis after sternotomy [23]. The hospital water system may be a potential reservoir for *Aspergillus* and other molds, which are then aerosolized [24]. No "safe levels" for bioaerosols have been recognized, and standards for the frequency of air sampling are also lacking. Rural outdoor air concentrations of fungi may be as high as 10,000 colony-forming units per cubic meter of air (CFU/m^3) without causing pulmonary infections in the general population. The establishment of a safe threshold limit in the indoor environment is problematic. Some studies have established a positive correlation between increased airborne spore counts and the incidence of invasive aspergillosis [25–27].

Researchers have collected air samples to quantify the number of airborne spores. Open agar plates, which are commonly referred to as "settle plates," should not be used to estimate the airborne concentration of fungal spores. The number of spores that settle on the agar due to the effects of gravity are presumed to be proportional to the airborne concentration, but are not reliable enough for routine use in facilities that perform organ transplants. Settle plates, however, may detect fungi aerosolized during medical procedures (e.g., during wound dressing changes), as described in an outbreak of aspergillosis among liver transplant patients [28]. Air sampling methods using calibrated sieve impactors or centrifugal samplers are recommended to provide standardized counts, the results of which are expressed as CFU per cubic meter. Routine air sampling for fungi is not generally recommended. During construction or renovation or in

times when isolates of *Aspergillus* or other fungi are identified in patient cultures, air sampling may be performed to assess the relative level of spores in the environment. Outdoor samples may be collected as appropriate controls. Fungal colony types found in the indoor samples should be the same as those from outdoor samples, but with a tenfold (1 log) reduction in indoor counts due to air-handler filtration [29]. Indoor samples that have a predominance of a particular fungus that is not in proportion to the outdoor samples may reflect contamination of the indoor environment.

46.1.6.2 Environmental Controls

The CDC guidelines for prevention of healthcare-associated pneumonia recommend protective environment units only for allogeneic HSCT recipient units; there are no specific recommendations for SOT recipients [2]. However, in 2003, the CDC published Guidelines for Environmental Infection Control in Healthcare Facilities which address specific controls applicable to all patients, and include recommendations specific to organ transplant recipients [3]. It is beyond the scope of this review to detail all recommendations in that guideline but several specific infection types are discussed. Facilities performing SOT surgeries should at minimum have contingency plans in case of disruption of HVAC services.

Specially designed isolation rooms that use laminar air flow (LAF) and/or high-efficiency particulate air (HEPA) filtration may provide the cleanest air possible. HEPA filtration, which provides a minimum of 12 air changes per hour, often reduces fungal spore counts. HEPA filters remove 99.97% of particles larger than 0.3 mm (micron). HEPA filters may be installed within the room ventilation system to provide a highly filtered, positively pressurized room, or portable units may be placed in any patient room for additional air filtration. Patient rooms should be tightly sealed to prevent contamination from outdoor sources, and their doors should remain closed to ensure positive pressurization. Reportedly, areas that use HEPA filtration and positive pressurization of patient rooms (fungal spore control ventilation) have total spore counts of less than 15 CFU/m^3, with *Aspergillus* counts of less than 0.1 CFU/m^3 [29].

Room design should focus on the use of easy-to-clean surfaces. The walls and horizontal surfaces should be smooth and nonporous to facilitate cleaning and to prevent entrapment of bacteria and spores. Porous ceiling tiles, carpeting, and fabric window treatments, such as shades and curtains, should be avoided as they may attract dust particles. Some new designs available are house curtains or shades within two glass panels which minimize dust collection while still providing privacy and controlling light. Vinyl or plastic blinds are safe if they are frequently cleaned.

Hospitalized SOT recipients should not travel through areas under construction or renovation. Severely immunocompromised patients requiring transport out of the protective environment should wear a high-efficiency respiratory protection mask, like N95, to prevent the inhalation of particulates [2]. Transplant recipients should also avoid dusty construction or excavation and landscaping sites after discharge. Historically, studies reported an association between the use of other protective isolation strategies, such as the restriction of fresh fruit and flowers with a decrease in the incidence of infection. The length of hospital stay is declining dramatically, so the benefits of a protective environment are being reevaluated. The most important risk factor for invasive aspergillosis remains the patient's underlying immunosuppressive condition. High-risk patients may develop invasive aspergillosis even with low fungal spore counts [25].

46.1.6.3 Construction Guidelines

Construction and renovation in the hospital are often associated with an increase in the number of cases of aspergillosis. At the beginning of renovation, airborne particulates and fungal spore counts may be exceptionally high because spores are dispersed into the environment during the demolition process. The Facility Guidelines Institute publishes guidelines for the design and construction of hospital and healthcare facilities [30]. Infection control personnel should be involved from the planning stages through project commissioning. Building owners are required to provide an infection control risk assessment (ICRA) to determine the potential risks of transmission of various infectious agents during the project. The ICRA is conducted by a panel with expertise in infection control and epidemiology, risk management, facility design, construction ventilation, and safety. An ICRA should be conducted during the early planning phase of the project, before construction begins, and continue through project construction and commissioning. Specific construction-related requirements mandated by the ICRA should be included in the contract documents. Many state health departments now require the ICRA submission before they will issue permits for hospital construction and renovation projects. When construction or renovation activities are planned in or near facilities that handle high-risk transplant recipients, even more strict protective guidelines and monitoring requirements may be established during the planning process [31]. Such guidelines help to define the appropriate barriers and techniques for preventing the spread of dust and debris into other areas of the facility. Construction and housekeeping personnel should be trained in the dangers of aspergillosis, with an emphasis on control measures. Strategies for the prevention of nosocomial aspergillosis will control any other fungi that are transmissible by dust, such as the zygomycetes (e.g., *Mucor* and *Rhizopus* species). Infection control interventions to prevent nosocomial aspergillosis were well illustrated during one construction-associated outbreak, in which the incidence of invasive aspergillosis rose from 3.18 to 9.88 cases per 1000 patient-days during the construction period [32]. The control measures that were used included portable HEPA filtration units, the installation of sealed windows and easy-to-clean tiles and shades, and the increased maintenance of the ventilation sys-

tem. The introduction of portable HEPA filter units was the most important step in this undertaking. After the institution of control measures, the infection rate decreased to 2.91 cases per 1000 patient-days.

When the construction activities are outdoors, the air intakes for the ventilation system may become heavily loaded with construction dust, potentially leading to an increased contamination of the indoor environment. An increased focus on filter maintenance is important, and successful containment may be possible, as a bone marrow transplantation unit reported during construction in its vicinity [31]. Maintaining the construction area at negative pressure, establishing plastic sheeting or drywall barriers, and controlling access to construction zones prevented dust from contaminating patient areas.

46.1.6.4 Surveillance for Fungal Healthcare-Associated Infection

If a case of nosocomial aspergillosis is suspected, it is crucial to look at the facility history of aspergillosis cases to assess background rates. An investigation of any ventilation deficiency is very important [2]. If there is a good chance that the case is healthcare-associated, then an epidemiologic investigation should be initiated in an effort to find and eliminate the source. *Aspergillus flavus* has frequently been identified in reports of construction-related contamination of the indoor environment [33]. Arnow et al. [34] reported an increase in spore counts of *Aspergillus fumigatus* and *A. flavus*, with a mean of more than 1 CFU/m^3 associated with the opening of a new hospital. An environmental assessment identified fungal contamination of the carpet, fireproofing material, and ventilation filters. Fungi may contaminate damp areas, discolored ceiling tiles, and peeling wallpaper. Most studies documented decreased indoor spore counts after the institution of appropriate control measures [31, 33]. Sometimes, air sampling is recommended for the assessment of air contamination after construction or HEPA filter changes and as part of an outbreak investigation. Repeat air samples may be collected after an identified source is decontaminated or removed. An environmental audit may also include periodic sampling. The role of fungal typing in the investigation of outbreaks is unclear; multiple fungal strains can cause healthcare-associated infections in one outbreak given the ubiquitous presence of fungi in the environment and the identification of different serial Aspergillus strains by whole genome sequencing within a single patient [35] may limit the application of this epidemiological tool.

Aspergillus species are certainly not the only significant fungal pathogen found in the environment. *Fusarium* and *Trichosporon* species, the dematiaceous molds, zygomycetes, and normally innocuous soil and plant fungi may cause infections in the immunocompromised patient. Good housekeeping practices are vital in high-risk patient areas. These areas should be visually monitored to ensure that all dust is contained and removed from the patient environment. If nosocomial infections occur within an institution, the renovation of ventilation systems to provide highly filtered air for high-risk patient areas may be considered. Although antifungal prophylaxis of patients may be useful, cases may still occur, necessitating the temporary closure of contaminated patient units or a suspension of transplant activities during hospital construction projects.

46.1.7 Waterborne Infections

Researchers at the University of Arkansas for Medical Sciences reported the results of a MEDLINE search of medical literature published from 1966 through 2001 to determine the number of HAIs caused by waterborne pathogens. Forty-three outbreaks had been reported, including many nosocomial outbreaks caused by *Pseudomonas aeruginosa* [36]. HAIs attributed to the use of contaminated water include those caused by *Legionella pneumophila P. aeruginosa; Aeromonas, Acinetobacter, Burkholderia, Enterobacter, Flavobacterium*, and other *Pseudomonas species; T. gondii*; and *Serratia*, Mycobacteria, and *Aspergillus* species. In 2006, the Environmental Protection Agency (EPA) published the Long Term 2 Surface Water Treatment Rule (LT2 rule), which addresses strategies to reduce disease incidence associated with *Cryptosporidium* and other disease-causing microorganisms in drinking water [37]. Individual state regulations or codes may identify requirements for maintaining hot water temperatures to protect patients from being scalded. Temperatures at the return should ideally be ≥124 °F (≥51 °C), and cold water temperature at <68 °F (<20 °C) in healthcare facilities [3].

46.1.8 Legionellosis

Transplant recipients are considered to be at increased risk of developing *Legionella* pneumonia, commonly known as Legionnaires' disease. Even after processing at water treatment plants, small quantities of these aquatic bacteria may enter homes and buildings and may live in the biofilm that lines the pipes. Legionella species multiply in warm water, with an ideal temperature range of 35–46 °C [38, 39]. Regulations concerning maximum water temperature, which are designed to prevent scalding accidents, often fall into this range, increasing the possibility that a facility will become contaminated with *Legionella* species and several other species of nontuberculous mycobacteria, including *Mycobacterium xenopi* [40]. Traditionally, it was believed that infection was caused by the inhalation of contaminated aerosols generated by humidifiers, air-conditioning units, cooling towers, and showers into the respiratory tract. The aspiration of contaminated water is an additional mechanism of transmission [41, 42]. Laboratory-confirmed Legionellosis in a patient who has spent ≥10 days continuously in a healthcare facility prior to the onset of illness is considered a definite case of healthcare-associated *Legionella*

pneumonia; that which occurs in a patient who has spent 2–9 days in a healthcare facility prior to the onset of illness is considered possible healthcare-associated Legionellosis [2, 3]. The 2003 CDC guidelines for the prevention of nosocomial pneumonia discuss issues of environmental monitoring and control. The recommendations state that facilities provide routine maintenance of their potable water systems and should consider the use of sterile water in immunocompromised patients if *Legionella* is isolated from the water to reduce the incidence of legionellosis [2].

L. pneumophila strains may be more virulent than the non-pneumophila strains. Advances in molecular fingerprinting techniques have been instrumental in associating patient isolates with Legionella species cultured from a facility's potable water supply. Furthermore, lawsuits have successfully linked nosocomial infection to perceived facility negligence [38].

46.1.8.1 Environmental Monitoring

Culturing plumbing fixtures, such as sink spouts, showerheads, ice machines, and drinking fountains, for *Legionella* species can identify potential sources of the bacteria in high-risk patient areas. The degree of contamination (percent of positive fixtures and quantity of bacteria present) varies significantly from building to building. The type of hot water system, the water temperature, location, and building age all play a role in the colonization of pipes within a facility [43]. The monitoring and control of *Legionella* species in a healthcare facility require a team effort, in which the microbiology laboratory, infection prevention and control, and maintenance departments must work together to provide a safe environment for high-risk patients. The CDC does not recommend routine environmental culturing for *Legionella*, but guidelines state that (1) this could be a component of *Legionella* prevention in healthcare facilities that provide care to transplant recipients, (2) may be appropriate to identify the source of infection as part of an outbreak investigation, and (3) to assess the effectiveness of water treatment or decontamination protocols [2, 3]. No guidelines regarding culturing frequency or acceptable levels of positivity are available. Generally, each facility will establish a policy on environmental monitoring that is dependent on the patient population. Environmental investigation to identify the source of *Legionella* is recommended when there is an outbreak, defined as one case of definite or two cases of possible healthcare-associated Legionnaires' disease within a 6-month period [3].

46.1.8.2 Legionella Control Measures in the Hospital

As a rule, if significant quantities of *Legionella* species are isolated in a facility, control measures to reduce the level of colonization should be instituted. Systems that use holding tanks or heaters that allow water to stagnate in the bottom of the tank provide a reservoir for the multiplication of *Legionella* species. For immediate control of Legionella in the setting of an outbreak, thermal eradication (superheat and flush) or hyperchlorination of the water supply is recommended [3]. Ongoing control of *Legionella* could be done with the use of copper/silver ionization systems, which release low concentrations of metal ions into the water distribution system, ultraviolet light sterilization, or maintenance of an elevated water temperature or chlorine content [44, 45]. Point-of-use filters have been found to be effective in eliminating *Legionella* and could be used without modification or disinfection of the potable water system [46], though are not on the current guidelines. In transplant units, shower heads and tap aerators should be removed, cleaned, and disinfected monthly using a chlorine-based, EPA-registered product; a 1:100 dilution of bleach may be used if no EPA-registered chlorine disinfectant is available [3]. In addition, large-volume room air humidifiers that generate aerosols should not be used unless they are subjected to high-level disinfection and only sterile water is used.

Even when control mechanisms are in place, healthcare-associated legionellosis may occur. Disruptions in the water distribution system, such as water main breaks, the use of fire hydrants, floods, and internal maintenance and construction disruptions, may cause changes in water pressure that disrupt the biofilm within the potable water system [38]. When pieces of the biofilm break free and enter the water supply, the water may appear cloudy or dirty. Local water authorities may issue water restrictions in the event of major contamination of the drinking water supply. Establishing water service disruption policies can be helpful for protecting immunosuppressed patients. Substitution of the appropriate bottled water is encouraged for drinking and for mouth care. Ice machine filters may become contaminated, so filters should be changed after restoration of water service [47]. Suspending showering until the water is determined to be safe may be necessary. When service is restored, all fixtures should be flushed until the water appears clear. Tub bathing may be acceptable because little aerosolization of the water occurs during the bathing process. Bed baths or other systems that do not generate aerosols are recommended.

The American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE) Standard 188p [48] establishes minimum risk management requirements to control legionellosis in water building systems, including inpatient healthcare facilities. An interdisciplinary designated team with the authority and responsibility to establish and implement a legionellosis risk management program, including but not limited to facilities staff familiar with the building water system and infection prevention and control staff should be formed. Components of the program include (1) description of the potable and nonpotable water systems in the building in water flow diagrams, including all water sources, water treatment systems and control measures, water processing, and end use points such as sinks, showers, water features, and ice machines, (2) identification of areas with higher probabil-

ity of infection based on the intended water use and the vulnerability to infection of patients in these areas, (3) identification of the control points where Legionella control measures can and should be put into place, (4) establishment of critical limits at the control points (e.g., temperature or chlorine level), (5) establishment of a monitoring system that includes the means, methods, and frequency for monitoring physical and chemical characteristics of the control measures to ensure they are within critical limits, (8) verification that the program is being implemented and validation that the control measures are effective in controlling Legionella, including a determination of if, when, where and how environmental cultures for Legionella are to be performed, and (9) documentation and communication of the plan.

46.1.8.3 Recommendations for the Discharged Patient

In areas where *Legionella* species have been identified in the water supply, patients who rely on well or spring water should be encouraged to have their own water supply checked [43]. One mistaken assumption is that all bottled water is safer or healthier than tap water; however, many water products are not processed to reduce bacterial contamination. Products such as spring water that emphasize natural properties may actually contain more bacteria than do other water products.

46.1.9 Antibiotic-Resistant Organisms: Vancomycin-Resistant Enterococcus, Methicillin-Resistant Staphylococcus, and Multidrug-Resistant Gram-Negative Bacteria

Infections caused by resistant organisms have emerged as a serious problem in hospitals all over the world. This is due in part to an increase in the nonselective use of broad-spectrum antibiotic agents for prophylaxis and treatment. The indiscriminate use of antibiotics reduces the normal host flora, predisposing the patient to colonization with endemic multidrug-resistant organisms and *C. difficile*. The emergence of *Streptococcus viridans* that is highly resistant to penicillin has been associated with the use of β-lactam antibiotics in neutropenic cancer patients [49]. Centers that routinely use quinolone prophylaxis for neutropenic patients have reported coagulase-negative *Staphylococcus* and gram-negative blood isolates that are resistant to these agents [50, 51]. HAIs with resistant gram-negative organisms (*Klebsiella species, Stenotrophomonas maltophilia,* and *Burkholderia cepacia*) are on the rise, a trend that may be related to the use of broad-spectrum antibiotics [52–54]. Gram-negative bacteria that produce extended-spectrum β-lactamases are becoming increasingly prevalent. Pretransplant broad-spectrum antibiotic use has been associated with post-transplant infections caused by multidrug-resistant organisms [55]. Antimicrobial stewardship programs and the use of local data to select appropriate treatments reduce the reservoir of multidrug-resistant pathogens within a medical facility. Reducing inappropriate antibiotic use by only prescribing an antibiotic when it is likely to be beneficial to the patient, minimizing the treatment of colonization, using broad-spectrum antibiotics judiciously, and discontinuing unnecessary and lengthy treatment with antimicrobials are the essence of antimicrobial stewardship. The CDC launched the "Get Smart for Healthcare" initiative to guide and support antimicrobial stewardship programs in different settings in order to improve antibiotic prescribing practices [56].

Patients who have longer inpatient stays before transplantation surgery may become colonized with multidrug-resistant organisms. Changes in the United Network for Organ Sharing (UNOS) allocation algorithm have resulted in an increased duration of preoperative hospitalization in some institutions. Data has demonstrated an increased number of cases of mediastinitis after heart transplantation caused by multidrug-resistant pathogens associated with an increased length of pretransplantation inpatient hospitalization prior to transplant [57].

Active surveillance identifies more patients colonized with resistant bacteria than clinical cultures alone, and this strategy can be used to control rates of colonization and infection due to resistant bacteria. The use of active surveillance for MRSA and subsequent decolonization with mupirocin has shown some effectiveness in reducing infections post liver transplantation [58] though some data have not demonstrated effectiveness [59]. Whereas the CDC guideline on the control of multidrug-resistant organisms recommends active surveillance for MRSA and VRE if other approaches have failed to control transmission adequately [4], the SHEA guideline recommends the use of active surveillance to identify patients colonized with MRSA and VRE among all high-risk patients [60]. Some states have mandated screening patients for MRSA. Active surveillance for MDR gram-negative organisms, including carbapenem-resistant enterobacteriaceae (CRE), is recommended in certain high-risk situations in order to prevent spread of these highly resistant and potentially virulent organisms [61]. Although active surveillance is not done routinely in most centers, the role of active surveillance for CRE in solid organ transplant candidates and recipients is unclear, and is an area worthy of future study.

46.1.9.1 Vancomycin-Resistant Enterococcus

Enterococci have become a significant infection control problem for decades, which is evidenced by the 20-fold increase in nosocomial infections reported to the NNIS from 1989 through 1993 [62]. Comparative data from the 1998 reports showed an additional 55% increase in VRE infections compared with that from 1993 through 1997. Between January 2006 and October 2007, 33% of enterococcal

device-related healthcare-acquired infections reported to the NHSN were caused by VRE [63]. The enterococci differ from other streptococci in their relative resistance to penicillins and cephalosporins and in their intrinsic low-level resistance to aminoglycosides and lincosamide antibiotics [64]. They are also resistant to bile, they are considered normal enteric flora in adults, and they generally exhibit low virulence. They may be isolated from the mouth, vagina, groin, and anterior urethra. The target of vancomycin in the cell wall is D-alanyl- D-alanyl, but, in VRE, this target is altered so that it has low affinity to vancomycin [65]. Using molecular typing techniques, VRE strains have been identified as comprising mainly three resistance phenotypes—van A, van B, and van C [64]. The van A phenotype is plasmid mediated, and, by definition, it is resistant to high levels of vancomycin and teicoplanin. The van B strains exhibit high-level resistance to vancomycin, but they are susceptible to teicoplanin. Class C shows constitutive low-level resistance to vancomycin; this is encountered in *Enterococcus gallinarum* and *Enterococcus casseliflavus*.

Risk Factors

Epidemiologic analysis has shown that enterococcal infection often originates from the patient's colonizing flora. Intraabdominal and cardiothoracic surgery and manipulation of the urinary tract are the risk factors for enterococcal infection. Reportedly, the severity of illness is one of the main risk factors for the development of VRE bacteremia [66–70]. Critically ill patients in the intensive care unit or those with underlying medical conditions, including immunocompromised patients residing on oncology and transplant units, are also at increased risk of colonization and infection with VRE strains. An increased length of hospitalization and antibiotic use contribute to the patient's risk [70]. Although vancomycin use is a predisposing factor for the acquisition of the organism, any antimicrobial agent that alters the normal gram-positive and anaerobic gut flora may allow VRE to flourish [66, 67]. In Europe, a glycopeptide (avoparcin) that is used in animal feeds has been associated with VRE in animals and humans. Antibiotics with antianaerobic activity have been shown to promote VRE high-density colonization both in animal models and in humans [70]. Both vancomycin and third-generation cephalosporins reportedly are independently associated with VRE prevalence in 126 intensive care units in the USA [71]. Reports have demonstrated the contact spread of the bacteria from patient to patient, both directly and indirectly via the hands of healthcare workers [72, 73]. Contaminated equipment and environmental surfaces are also sources for disease transmission [74].

In the setting of solid organ transplantation, most VRE infections occur in the early post-transplant period, with a strong association with antimicrobial use and surgical, specifically biliary, complications. Liver transplant recipients who developed VRE bacteremia were compared retrospectively with transplant recipients who developed bacteremia with vancomycin-sensitive enterococci (VSE) [75]. VRE infection was associated with increased episodes of recurrent bacteremia and persistent isolation of the bacteria from the original site of infection. Whether VRE strains are more virulent than VSE is still a controversial issue, but, in that study, few cases of endovascular infection were encountered among the VRE patients and none among the VSE control patients.

The VRE colonization rate of patients awaiting liver transplantation was reported to be 13% [76]. Another 18% became colonized after transplantation. Infection with VRE occurred in 23% of these patients. A recent meta-analysis demonstrated that post-transplant MRSA and VRE colonization was significantly associated with post-transplant MRSA and VRE infection [77]. Patients who were colonized with VRE either before or after transplantation had longer hospital and ICU stays. Those that acquired VRE after transplantation also had higher 90-day mortality.

The fecal carriage of VRE has also been studied in an outbreak on a renal unit. The authors used restriction enzyme analysis and ribotyping to show that the outbreak isolate was clonally related [70]. VRE was isolated from the stool of 15% of renal patients (i.e., those with end-stage renal disease), 5% of other patients in the hospital, and 2% of sampled patients in the community with no history of hospitalization or antibiotic use. Many studies have used DNA analysis to show that nosocomial transmission is the primary route of VRE colonization among patients.

Infection Prevention and Control Measures

The CDC Hospital Infection Control Practices Advisory Committee developed guidelines for preventing the spread of VRE [78]. The following four main points are crucial for prevention: (1) prudent vancomycin use, (2) an education program, (3) an effective microbiology laboratory, and (4) a multidisciplinary effort to control the organism.

The microbiology laboratory initiates the process of VRE control by promptly and accurately identifying the organism. Vancomycin resistance can be identified through routine bacterial susceptibility testing or through polymerase chain reaction (PCR) testing using primers to detect the vanA and vanB genes. When a vancomycin-resistant strain is identified, the infection control department, the patient's physician, and unit personnel should be notified. Those patients who are colonized or infected should be placed in single rooms, or they may be cohorted with other VRE-positive patients. Because the bacteria may colonize the intestinal tract, patients with poor personal hygiene or fecal incontinence may contaminate the environment with the bacteria. Patients may also contaminate their immediate environment by touching surfaces, such as bed rails, nurse call buttons, and television controls. This type of equipment may not be adequately disinfected after the patient leaves, increasing the risk of transmission for the next patient. The recommendation is that gloves and gowns should

be worn when one is entering the room of colonized patients, especially in endemic settings. Some groups report significant decreases in VRE infection and VRE colonization after the institution of enhanced infection control measures in conjunction with judicious restriction of certain antibiotics, such as vancomycin and third-generation cephalosporins [79, 80].

Various reports have documented the isolation of VRE strains from environmental surfaces [72, 73, 81]. Noncritical items, such as stethoscopes or thermometers, should not be used with other patients unless they are thoroughly disinfected after use for a VRE patient. Dedicated equipment is preferred, but it may be shared among cohorted patients. Patients may be screened for VRE carriage by the collection of rectal swabs or stool for cultures or PCR testing to identify additional cases. This information is useful for determining transmission between roommates or to others on a unit where infected patients have been identified. Despite the institution of contact precautions for carriers, the incidence of carriage may remain about the same [69]. VRE colonization may persist for long periods [82]; therefore, colonized or infected patients may require continuous isolation until their discharge. If a patient is being transferred to another facility, notifying the receiving institution about the patient's VRE status so that the appropriate precautions are taken is imperative. VRE-positive patients who are readmitted should be placed in contact isolation until surveillance cultures have been completed.

Concerns that a plasmid that carries the vancomycin resistance gene could transmit this resistance to other gram-positive bacteria, particularly *S. aureus*, do exist. Because of the multiple virulence factors associated with this pathogen, these infections would potentially be life threatening because the organism is already resistant to multiple antimicrobial agents. Vancomycin-resistant *S. aureus* (VRSA) has been isolated. Seven cases of VRSA were identified in the USA from 2002 to 2006. All isolates were vanA positive. All patients had a prior history of MRSA and enterococcal infection or colonization. They all had severe underlying conditions and most had received vancomycin prior to VRSA infection. Proper isolation precautions were in place and prevented person-to-person transmission in all seven cases [83]. As of 2014, 13 cases of VRSA have been identified [84].

46.1.9.2 Methicillin-Resistant S. aureus

MRSA is a well-recognized nosocomial pathogen causing significant infections in all patient populations. Contact precautions are used to isolate patients with MRSA infection or colonization. Some controversy exists over the use of masks to enter MRSA patient rooms. Because patients with nasal colonization may spread the organism into the surrounding air, some advocate that caregivers don masks to prevent their acquisition of the organism, thus minimizing spread to other patients. Transmission on the hands of colonized staff members may be increased if they touch their noses during patient care activities.

Risk Factors

As the Temple University experience illustrates [85], MRSA colonized patients are more likely to infect their surgical wounds. Researchers in a French study collected surveillance cultures from liver transplant recipients. The analysis of the infection data found that MRSA infection occurred more frequently in the MRSA carriers (7 of 8 patients, 87.5%) than in the MRSA noncarriers (8 of 79 patients, 10.1%) ($P<0.001$) [86]. Among liver transplant recipients, patients who underwent surgery within the prior 2 weeks were at markedly higher risk for MRSA infection [87]. A review of infections occurring from 1990 to 1998 in another liver transplant center in the USA showed that 23% of organ recipients became infected with MRSA, with significant increases in the incidence and prevalence of patients infected with MRSA over time [88]. The primary sites of infection were the vascular catheter (39%), the wound (18%), the abdomen (18%), and the lung (13%). CMV seronegativity ($P=0.01$) and primary CMV infection were significantly associated with MRSA infections ($P=0.005$). Although relatively uncommon, donor-derived MRSA transmission has been described following liver transplantation [89].

Infection Prevention and Control Measures

Quality improvement programs should be aimed at reducing HA-MRSA acquisition and infection rates and a multifactorial approach towards decreasing MRSA transmission has been described [90]. The collection of surveillance cultures may be cost-effective in all patient populations, and the high-risk transplant recipient group may be an ideal starting point for the process. An MRSA control program involving liver transplant patients consisting of active surveillance, use of contact precautions and cohorting of colonized patients, treatment with intranasal mupirocin at the time of transplantation, and education of patients and visitors on the importance of hand hygiene resulted in a decrease in the incidence of new MRSA colonization, MRSA bacteremias, and MRSA infections at other sites [58].

46.1.9.3 Multidrug-Resistant Gram-Negative Bacteria

There is no standard definition for multidrug-resistant gram-negative bacteria (MDR-GNB). Included in this category are bacteria resistant to multiple classes of antibiotics, such as *P. aeruginosa* and *Acinetobacter baumanii*, as well as Enterobacteriaceae (e.g., *Escherichia coli* and *Klebsiella pneumoniae*) with extended-spectrum β-lactamases (ESBLs) that hydrolyze β-lactam antibiotics including extended-spectrum cephalosporins [4]. There has been a substantial increase in MDR-GNB, defined as being resistant to three or more antimicrobial classes [91, 92]. *A. baumanii*, which is frequently resistant to multiple antibiotics including β-lactams, fluoroquinolones, and aminoglycosides, is becoming increasingly resistant to carbapenems, and isolates resistant to all

tested antibiotics have been reported [93]. Patients with infections due to MDR-GNB are more likely to experience delay in institution of effective antimicrobial therapy, have a higher mortality, and increased cost of care [94, 95].

Extended-spectrum β-lactamases are usually found in *K. pneumoniae, K. oxytoca*, and *E. coli*, but have also been reported in *Citrobacter, Enterobacter, Proteus, Salmonella, Serratia*, and other gram-negative bacteria [96]. Gram-negative bacteria with ESBLs are typically sensitive to the carbapenems, which are recommended as treatment for infections due to these organisms. Recently, *K. pneumoniae* with a carbapenem hydrolyzing enzyme, which confers resistance to all carbapenems, have been reported, and this organism caused 8% of device and surgery-associated HAIs [63, 97]. Though named KPC (*K. pneumoniae* carbapenemase) as this was initially found in *K. pneumoniae*, KPC has been reported in other enterobacteriaceae, including *E. coli, Enterobacter* species, and *Serratia* [94, 98]. There is variation in the geographic distribution of ESBL-containing organisms. While it occurs sporadically in various states, *K. pneumoniae* with KPC has become endemic in the eastern United States and spread throughout the USA is increasing [99]. Infections caused by carbapenem-resistant enterobacteriaceae are associated with high mortality rates among liver transplant recipients [100].

Resistance genes in gram-negative bacteria could be chromosomal, or could be located in mobile genetic elements, such as plasmids and transposons, which can be transferred between different species [101]. Some of these gene elements may contain multiple genes encoding resistance to penicillins, cephalosporins, carbapenems, and aminoglycosides conferring multidrug resistance [102]. Quinolone resistance is usually due to chromosomal mutations and not usually transferable, but transferable quinolones resistance genes encoded on plasmids have been identified, and recently, MDR-*K. pneumoniae* with a plasmid containing resistance determinants for carbapenems, aminoglycosides, and fluoroquinolones was reported [103].

Risk Factors

Risk factors for colonization or infection with MDR-GNB are similar to those for MRSA and VRE and include advanced age, underlying diseases and severity of illness, transfer of patients from another institution particularly from a nursing home, prolonged hospitalization, gastrointestinal surgery or transplantation, presence of invasive devices such as CVCs, and exposure to antimicrobial drugs [104]. Prior solid organ or hematopoetic stem cell transplantation has been identified as a risk factor for infections caused by carbapenem-resistant *K. pneumoniae* [105]. Bacteremia due to a KPC-2–producing *Enterobacter cloacae* and *Pseudomonas putida* has been reported in a liver transplant recipient [106].

Automated susceptibility testing systems have limitations in detecting drug resistance in these organisms [97, 107]. Providers and clinical microbiology laboratories should be familiar with these organisms and ensure that organisms are tested using methods that will provide reliable susceptibility results.

Hospital outbreaks due to MDR-GNB have been reported [97, 108]. Similar to MRSA and VRE, many more patients may be colonized than infected, providing an unrecognized reservoir, and active surveillance screening may be necessary to prevent cross-transmission.

Infection Prevention and Control Measures

Measures to control the spread of MDR-gram-negative bacteria are similar to other drug-resistant organisms which include (1) administrative support such as instituting automatic alerts and provision for adequate hand hygiene facilities, (2) education of personnel regarding MDROs and prevention methods, (3) judicious antimicrobial use, (4) surveillance, (5) contact precautions, and (6) enhanced environmental cleaning. Published guidelines have specifically addressed infection control guidance for the prevention of infections caused by carbapenem-resistant organisms which addressees active surveillance of high-risk units and contacts with infected patients [61].

46.1.9.4 C. difficile Infection

Numerous factors may cause diarrhea in transplant recipients, including immunosuppressants, antibiotics, enteral nutrition, and other agents that affect bowel motility. The extended use of antimicrobials alters the bacterial flora of the gut, providing a niche for the multiplication of *C. difficile*, an anaerobic, spore-forming, gram-positive rod that is resistant to many antimicrobial agents. Although the organism occurs as normal enteric flora in approximately 4% of adults, it may also cause severe gastroenteritis that manifests as either diarrhea or colitis. *C. difficile* produces the following two toxins: toxin A, or enterotoxin, and toxin B, or cytotoxin. These toxins act synergistically, resulting in cellular damage, hemorrhage, and the accumulation of fluid in the colon. Most patients have a history of antibiotic usage before the onset of diarrhea. *C. difficile* is the most common cause of healthcare-associated diarrhea, with higher rates of carriage, ranging from 15% to 30%, reported in hospitalized patients [109]. *C. difficile*-associated diarrhea occurs in 1–31% of SOT recipients [110].

In 2011, there were an estimated 453,000 cases of *C. difficile* disease (CDD) in the USA resulting in over 29,000 deaths [111]. One specific strain (NAP1/BI/027) has emerged that is more virulent and more resistant to antibiotics, particularly fluoroquinolones. It produces more toxin A and toxin B, and produces a third toxin, binary toxin. The disease is more severe and has affected patients with no underlying risk factors for CDD [112]. Outbreaks have been associated with fluoroquinolone use, though other antimicrobials have also been implicated [113, 114].

Risk Factors

Although any antibiotic agent can affect the normal balance of the intestinal flora, clindamycin, penicillins, fluoroquinolones, and the third generation cephalosporins have been particularly associated with the development of infection [115, 116]. Other factors that alter the gut flora also increase the risks of carriage of the organism in the bowel and of disease. The use of stool softeners and antacids has been associated with increased carriage [117]. Diarrhea has also been associated with older age, underlying disease, and enemas. Symptomatic patients usually have more risk factors, and certain intrinsic patient factors influence the relative risk of developing symptomatic infection [118]. In solid organ transplantation, most cases of CDD occur in the first 3 months of transplant, likely attributable to the increased rates of hospitalization and use of antibiotics during this time period [119].

46.1.9.5 Healthcare-Associated Transmission

Documented clusters of cases due to healthcare-associated transmission have frequently been associated with environmental contamination with bacterial spores. In one study, 21% of patients who were initially culture-negative admitted to a general ward acquired *C. difficile* while in the hospital; of these, 37% developed diarrhea [120]. The authors were able to prove transmission between patients with the use of an immunoblot technique, and they documented clustering in patient rooms with two occupants. Other authors have suggested other patterns of acquisition in situations in which no evidence of transmission to roommates exists [121]. One cluster investigation identified two case strains of bacteria by restriction endonuclease testing, in which most of the strains were associated with abdominal surgeries performed by one surgical team [122].

Because of spore production, this organism can survive well in the environment. *C. difficile* has been cultured from inanimate objects, such as medical instruments, toilets, bathroom floors, and furniture [123]. Bacteria have been cultured from the hands of medical personnel, and strains isolated from medical staff caring for patients with *C. difficile* were confirmed to be the same as those of the patient isolates [120].

Patients may become colonized with *C. difficile* via transmission through contact with other patients, contaminated rooms or equipment, or medical personnel carrying these bacteria on their hands. More environmental contamination with spores occurs in the room of a patient who has CDD, than with those with asymptomatic carriage. Nosocomial attack rates vary from facility to facility. Clinicians caring for transplant recipients should therefore be aware that nosocomial transmission of *C. difficile* is a real possibility and that the early implementation of infection control measures may prevent the occurrence of other cases.

Detailed strategies for the prevention of *C. difficile* in hospital settings have been published [124]. Patients should be placed in private rooms or cohorted with other infected patients. Symptomatic patients should be placed on contact precautions. Healthcare personnel should wear gloves and gowns when they enter the patient's room. Patient transport outside the unit should be minimized if the patient has diarrhea to avoid contaminating other areas with the bacterial spores. Good hand hygiene is essential. Soap and water or alcohol hand sanitizer may be used in routine or endemic settings; soap and water is preferred for outbreak or hyperendemic settings. Staff members must observe proper procedures, and visitors should be encouraged to wash their hands thoroughly before leaving the patient's room. A dilute (1:10) hypochlorite solution or a product with an EPA-approved claim for *C. difficile* activity should be considered in units with high *C. difficile rates* [6]. During outbreaks, environmental decontamination, isolation or cohorting of infected patients, and the limitation of clindamycin have significantly reduced CDD [125]. The use of dedicated patient equipment or of disposables, such as rectal thermometers, may significantly reduce the incidence of CDD in both acute and chronic care facilities and should be used whenever possible [126].

46.1.9.6 Antimicrobial Therapy Issues

Although the initial step in the treatment of *C. difficile* is the discontinuation of the antibiotic agent(s) to allow the recolonization of the gut with normal flora, oral metronidazole or oral vancomycin is often used to treat the infection [127]. A randomized double-blinded placebo-controlled study shows that patients with mild to moderate disease respond comparatively with either metronidazole or oral vancomycin. Patients with severe disease, however, had better clinical cures with vancomycin [128], and so vancomycin should be considered as the first-line agent for patients with severe disease. Fecal microbiota transplantation has emerged as a promising treatment for patients with frequent relapses of CDAD [129], however, its safety and efficacy in the setting of organ transplantation is unknown. Guidelines recommend against antimicrobial prophylaxis for patients at risk for CDD, treatment of asymptomatic carriage, and test of cure [124].

46.1.9.7 Outcomes

CDD has been reported to follow a more fulminant course in transplant recipients. A retrospective study of severe CDD showed that 13% of lung transplant patients had fulminant symptoms compared to 1.6% of all patients with CDD. Of those who required colectomy, 27% were transplant recipients, mostly lung transplants, though they actually had a better survival than nontransplant recipients [130]. The authors stated that improved awareness, lower threshold for surgery,

and closer follow-up may have paradoxically improved the outcome in transplant recipients. A review of cystic fibrosis patients showed that those who had lung transplants tended to have a more complicated disease course [131]. Other studies did not show more complicated disease, relapse rates, or mortality from CDD in SOT recipients [132, 133].

46.2 Community-Acquired Infections

46.2.1 Tuberculosis

Overall TB incidence rate in the USA has been declining, but TB in foreign-born and racial/ethnic minorities has been much higher compared to US-born Caucasians [134]. The proportion of cases in foreign-born persons has been increasing and accounted for 66% of cases reported in 2014, with Mexico, the Philippines, India, Vietnam, and China as the top 5 countries of origin [135]. Most cases of TB in foreign-born patients represented the reactivation of TB that had been acquired in the country of origin [136]. Between 1983 and 1994, the authors treated 14 liver transplant recipients (0.5%) for active TB; the most important risk factor was birth in a foreign country with endemic TB [137].

Multidrug-resistant (MDR) TB, defined as resistant to at least isoniazid and rifampin, appeared in the USA and worldwide in the 1990s, and required treatment with second-line anti-TB medications [138]. In the USA, the proportion of patients with MDR TB in those without previous TB has remained stable at 1% from 2009 to 2013. The percentage of MDR TB has remained below 1% in US-born cases, but of the total number of reported primary MDR TB, the proportion occurring in foreign-born cases increased from 30.8% in 1993 to 89.5% in 2013, accounting for 92% of primary MDR TB US cases in 2013 [135]. The CDC and World Health Organization (WHO) surveyed laboratories across the world and found that between 2000 and 2004, 20% of TB isolates were MDR and 2% were extensively drug-resistant (XDR) TB. The provisional definition of XDR-TB was an isolate resistant to isoniazid and rifampin and at least three or more of the six main classes of second-line drugs [138]. The definition was revised in October 2006 and XDR-TB is that which is resistant to isoniazid and rifampin, and resistant to any fluoroquinolone and at least one of three injectable second-line drugs (amikacin, kanamycin, and capreomycin) [139]. From 1993 to 2011, 63 such cases were reported in the USA, 17 of which occurred in 2000–2006 [140].

TB prevalence in the community and the presence of MDR- and XDR-TB [141] heighten the possibility that an immunosuppressed individual will be exposed to a case of active TB. Transplant recipients are susceptible to infection with *M. tuberculosis* species, and the progression to active disease can be quite rapid, similar to the experiences of patients infected with HIV [142]. In April 2007, three patients received organs from a 46-year-old US-born man with history of seizure disorder, alcoholism, homelessness, and incarceration, who was initially hospitalized for presumed aspiration pneumonia [143]. The two kidney recipients developed fever 6–7 weeks after transplant and cultures grew *M. tuberculosis* which matched the donor's *M. tuberculosis* isolate which grew from CSF postmortem.

46.2.1.1 Isolation

In the transplant unit, instituting appropriate isolation as quickly as possible when active TB is a possibility is essential. Patients who have an increased potential for TB should be placed in isolation rooms if active disease is even a remote possibility. In a 1990 nosocomial outbreak of TB that occurred among renal transplant recipients, the disease was transmitted from the source patient to five other patients on the same unit. The institution of airborne precautions was delayed because the TB infection had an atypical presentation in this patient [144]. Restriction fragment length polymorphism analysis confirmed the strain. Mortality in this patient group was 50%, with the shortest incubation time between exposure and active infection being approximately 5 weeks.

It is important to have a high index of suspicion for TB disease, in order to prevent transmission by prompt placement of suspect patients in an airborne infection isolation (AII) room until active TB is ruled out [145]. Airborne precautions can be discontinued when infectious TB is considered unlikely and either (1) another diagnosis is made that explains the patient's illness, or (2) 3 sputum specimens collected at 8-hour intervals, at least one of which is collected in the early morning, are AFB smear-negative. The Xpert MTB/RIF assay is an FDA-approved nucleic acid assay (NAA) that detects the presence of TB and rifampin resistance in sputum specimens; the negative predictive value for the presence of AFB smear positive TB is 99.7% after 1 negative assay and 100% after 2 negative assays [146]. CDC recommendations of 3 negative sputum specimens prior to discontinuation of airborne precautions in patients with suspected TB allow for the use of AFB smear, or NAA, or a combination of the two [147]. For patients with TB, decisions regarding discontinuation of AII require 3 negative sputum smears and clinical criteria.

The CDC guidelines describe ventilation system requirements for TB isolation rooms [145]. These include engineering controls to contain any droplet nuclei to prevent dissemination outside the patient's room. The AII room must be at negative pressure to the corridor, and exhaust air must be vented to the outside of the building or filtered through HEPA filters before it is recirculated. The AII room should have a permanently installed visual mechanism to monitor the pressure differential between the room and the corridor when occupied by patients with suspected or confirmed TB [30]. An ultraviolet germicidal irradiation device can be installed to irradiate the air in the conduit so bacteria are inactivated. There should be more than or equal to six air

changes per hour. New or renovated healthcare facilities should construct the AII room with more than or equal to 12 air changes per hour. Facilities may have to replace or retrofit their ventilation system to fulfill the safety criteria. This ventilation design is also required for varicella isolation. All employees must use a National Institute for Occupational Safety and Health (NIOSH)-approved fit-tested N95 respirator or powered air-purifying respirator when they enter the room of a patient with active or suspected TB.

46.2.1.2 Diagnosis

Active infection in the transplant recipient may not present with the traditional symptoms found in the general population [85]. Pulmonary infiltrates or pleural effusion may constitute TB infection without the other typical symptoms. Many transplant candidates with terminal organ failure are anergic, and after transplantation, most recipients remain or become anergic secondary to immunosuppressive agents that are administered to prevent organ rejection. Therefore, the use of the tuberculin skin test (TST) to monitor transplant recipients rarely provides useful information. Important information is provided when conversion from negative to positive tuberculin skin testing occurs, but negative results do not rule out infection. Furthermore, the value of the anergy testing is in question and is no longer recommended to be done routinely [145, 148]. Even when the patient reacts to one of the other antigens and his or her TST test is negative, the patient may still have latent infection or even active TB. Disseminated TB occurs more frequently in transplant recipients because the major host defense against TB is cell-mediated immunity [85].

TST testing of close family members may provide additional information on the patient's potential to spread this infection. In recent years, in vitro interferon gamma release assays (IGRAs) became available for diagnosis of latent TB (QuantiFERON TB test (Qiagen, Germany) and T-SPOT.TB test (Oxford Immunotec, UK)). These tests measure γ-interferon production when lymphocytes are incubated with synthetic peptides that simulate some proteins present in MTB [149, 150]. These tests are more specific than TST for detection of MTB infection, with much less false positive results related to previous BCG administration and previous exposure to atypical mycobacteria. Both tests are approved by the U.S. Food and Drug Administration. The CDC recommends using these tests for the same indications as the TST [145].

Fluorescent microscopy for the evaluation of acid-fast bacillus (AFB) smears and radiometric culture methods provide important diagnostic information, which may lead to the earlier initiation or discontinuation of patient isolation. Many microbiology laboratories now use rapid testing methods to detect and confirm TB, including nucleic acid amplification tests for detection of MTB in smear-positive and smear-negative respiratory specimens and DNA probes for species identification [151].

46.2.1.3 Post-exposure Follow-Up

If transplant recipients are exposed to a person with active TB, a contact investigation should be initiated. Recent TST or IGRA results and chest radiographs taken before the exposure may be used for baseline data. Additional TST testing should be performed 8–10 weeks after the exposure to evaluate for skin test conversion. Prophylactic isoniazid (INH) therapy should be considered for the prevention of the disease if the exposure is considered significant. If the source patient has a strain of TB that is either documented or suspected to be drug resistant, the use of alternative prophylactic regimens should be considered [152]. Prophylactic regimens for multidrug-resistant TB are not well established. In the past, the authors have used pyrazinamide with levofloxacin after such an exposure occurred among organ transplant recipients [153]. This regimen was associated with a high rate of discontinuation of the medication due to the adverse drug effects.

An international debate regarding the use of BCG vaccine for the prevention of TB spread has been ongoing. In the USA, indication for its use rarely exists [154]. Disseminated BCG disease is a risk in immunocompromised patients, including transplant recipients.

In June 2000, the CDC and the American Thoracic Society (ATS) formulated some new recommendations regarding TB prophylaxis and introduced two terms [155]. The first term is "targeted tuberculin testing" (i.e., TB testing by TST placement of patients at high risk for the development of TB). The second term is "latent TB infection" (i.e., patients who have been infected with TB but who have not developed TB disease). Chemoprophylaxis or preventive therapy is termed the "treatment of latent TB infection."

A skin induration of 5 mm or more after TST testing is considered positive for the following patient categories: patients infected with HIV, patients receiving immunosuppressive agents including transplant recipients, patients with recent contact with active TB, and patients who have an abnormal chest radiograph that is consistent with old TB. These patients are at high risk and are candidates for treatment of latent TB [145].

Three regimens for the treatment of latent TB infection exist. These are as follows:

1. INH for 9 months
2. Rifampin for 4 months
3. INH and rifapentine for 12 weeks

INH and rifapentine are convenient as the long half-life of rifapentine allows for once weekly dosing; the CDC recommends directly observed treatment with this regimen [156]. The clinician should note, however, that these regimens could have risks when used in SOT recipients. Cases of severe INH hepatitis have been reported [157]. Severe liver injuries, resulting in the death of five patients, occurred as a result of the rifampin–pyrazinamide combination [158] and it is no

longer recommended. Rifampin has also been associated with the severe rejection of solid organs, due to its interactions with cyclosporine and tacrolimus [159].

46.2.2 Varicella-Zoster Virus

Varicella is a highly infectious virus and up to 90% of seronegative household contacts may become infected after infection in the family.

46.2.2.1 Isolation

The infection control management of VZV in the immunosuppressed population involves some difficult issues. Varicella is transmitted via the airborne route during the primary infection (chickenpox) as virus particles are released from the airways of the patient into the environment. Transmission may also occur after direct contact with moist vesicles. Transmission of the virus usually starts 1–2 days prior to rash onset and lasts until the skin lesions are crusted. CDC guidelines require both airborne and contact precautions for patients with varicella and disseminated zoster [5]. One of the most difficult tasks for infection control is to define "disseminated" zoster for instituting room isolation. In localized dermatomal zoster, transmission occurs primarily through direct contact with the skin lesions, and only standard precautions are necessary.

In the immunosuppressed host, even small number of moist lesions and possibly respiratory secretions may contain enough viral particles to transmit the infection to other susceptible individuals through airborne routes or the shedding of the viral particles from skin lesions into the surrounding air. An adult cadaveric renal transplant recipient who occupied a private room adjacent to a patient with zoster developed fatal hepatitis after the nosocomial transmission of primary varicella infection [160]. Using PCR, Sawyer et al. confirmed VZV DNA in 82% of air samples collected in varicella patient rooms and in 70% of air samples collected in zoster patient rooms [161]. In a few samples, the virus was detected outside the door of negatively pressurized isolation rooms. Although this may represent a failure of the ventilation system to maintain negative pressurization of the room or of staff members leaving the door to the room open, obviously aerosolization of the viral particles does occur. The virus was also detectable up to 6 days after the onset of rash with the use of the same technique.

46.2.2.2 Patient Screening

Varicella infection in susceptible immunosuppressed patients may result in visceral disease, and it is associated with high mortality. In a series of three adult liver transplant recipients who developed varicella hepatitis, one patient died after developing adult respiratory distress syndrome and disseminated intravascular coagulation [162]. The introduction of a vaccine has significantly reduced varicella-zoster morbidity and mortality. Its use has been expanded since 1999 to include HIV-infected children with CD4 percentage of 15–24% and adults with CD4 count of at least 200 cells/μL [163]. There are two vaccines currently available: Varivax, a single-antigen varicella vaccine, and ProQuad, a combination of varicella and MMR vaccines. The latter contains more virus than the former vaccine [164]. Varicella vaccine is contraindicated in the transplant recipients, because it is made from a live, attenuated virus. However, the experience with leukemic children has shown that the vaccine is safe and effective [163]. Some reports have also demonstrated that the live, attenuated vaccine is safe and efficacious in susceptible pediatric kidney transplant recipients [165]. Researchers in that study administered the vaccine at candidacy; the results showed a reduced incidence of varicella after transplantation. There are also some reports of vaccination after transplantation. In one of these, seroconversion occurred in 20 of 31 (64.5%) children; 7 required multiple doses and only minor local skin reactions were observed [166]. The risk that healthy individuals will develop a rash after vaccination and transmit it to an immunosuppressed patient is low, and, therefore, vaccinating susceptible individuals, including healthcare workers, living in the same household with transplant recipients is not contraindicated [164]. The vaccine manufacturer does recommend that healthcare workers who develop vesicles should not care for susceptible individuals. Although some have hypothesized that the vaccine strain of virus may not be capable of causing secondary infections, a few such cases have been documented [167, 168]. The general consensus is that the benefits of vaccination of household contacts of immunocompromised individuals outweigh the very low risk of transmission of the vaccine virus to the transplant recipient. Varicella vaccine is also used in healthy persons as a post-exposure preventive measure, mostly in unvaccinated children, ideally within 3–5 days after exposure [169].

46.2.2.3 Post-exposure Management

Transplant coordinators must frequently evaluate the exposure of a transplant recipient to an individual with "possible" chickenpox. Most commonly, the exposure occurs after contact with a family member, usually a child. Defining the nature of the exposure by duration, proximity, and disease progression is an important step in the assessment process. Direct exposure is one that occurred face-to-face indoors. The duration of significant exposure is not clear; some experts say exposure for more than 5 min is significant, though others state that more than an hour is needed [163].

Documentation concerning each patient's varicella-zoster immune status must be easily accessible. Most adult patients are seropositive for VZV even if they do not recall having had chickenpox. After exposure to a patient with VZV infection the CDC's Advisory Committee on Immunization Practices

(ACIP) currently recommends VariZIG administration be considered for seronegative immunocompromised patients and certain other groups such as pregnant women and their newborns, for whom complications of disease could be life threatening [170].

VariZIG is expected to provide maximum benefit when administered as soon as possible after exposure, although it can be effective if administered within 10 days after exposure [170]. Although breakthrough infection after varicella-zoster immunoglobulin (VZIG) administration was common, its use ameliorated the severity of the disease. In one study in a SOT pediatric population receiving VZIG (median age of 8 years), 55% developed varicella, but only 4% developed severe disease [171]. The usual dosage is 125 units for each 10 kg of weight, up to a maximum of 625 units. If another exposure occurs more than 3 weeks after the administration of the VZIG dose, an additional dose of VZIG should be administered to provide continued passive immunity [169]. Patients who get monthly high-dose IVIG (>400 mg/kg) are protected if the last dose was given less than 3 weeks before exposure [161]. Because varicella immune globulin could prolong the incubation period by ≥1 week, patients given VariZIG should be monitored for signs or symptoms of varicella for 28 days after exposure. Antiviral therapy should be started as soon as signs or symptoms of varicella occur. Acyclovir may also prevent or attenuate infection after VZV exposure and may constitute a valid alternative, especially in those cases that come to medical attention more than 10 days after exposure. Some authors have advocated using it with VZIG in cases where life-threatening VZV infection is possible, such as in children with renal disease who are receiving steroids [172]. Acyclovir is FDA approved for the treatment of varicella in healthy children. The American Academy of Pediatrics (AAP) recommends consideration of acyclovir treatment of individuals at risk of moderate or severe varicella [173]. The value of acyclovir as a prophylactic agent in the immunocompromised host is unclear; VariZIG is recommended after exposure of these individuals to VZV. There is limited data to support the use of acyclovir for post-exposure prophylaxis in healthy children [174] though some experts support this approach in immunocompromised patients, particularly if VariZIG is unavailable.

46.2.2.4 Staff Considerations

Not all susceptible healthcare workers who report exposure to VZV develop chickenpox. In one report, the incidence of varicella after exposure approached 10% [175]. Susceptible healthcare workers who report such exposures should be furloughed from work from the 8th to the 21st day after exposure. This is based on the average incubation period of 14 days and the knowledge that transmission may occur up to 5 days before and 6 days after the onset of the rash [176]. Susceptible healthcare workers who are exposed to VZV put their transplant recipients at risk, but their furloughed absences also have cost implications and cause disruptions of patient care [177]. Therefore, CDC recommends the vaccination of susceptible healthcare workers if no contraindications are identified [178]. Healthcare workers who receive acyclovir prophylaxis may exhibit a longer incubation period before the development of a rash. Maintaining accurate records of employee data concerning vaccination or a previous history of chickenpox is important. Susceptible employees should be actively encouraged to receive varicella vaccine.

46.2.3 Respiratory Viruses

Most respiratory tract viral infections are seasonal, are more prevalent in children than in adults, and are transmitted by droplets rather than aerosols. Coughing, sneezing, or talking may generate droplets that are not usually projected farther than 3 ft (0.9 m) from the source patient. Special ventilation is not required for inpatient isolation. In the hospital setting, suctioning respiratory secretions and performing bronchoscopy may also generate droplets. The most common respiratory viral infections include RSV, influenza, parainfluenza, and adenovirus. The infection control aspects of respiratory viral infections are similar, and RSV is described here as an example. In recent years, the importance of these viruses in SOT recipients has received more recognition, as has the realization that these viruses cause significant morbidity [179]. These viral infections could be followed by superinfection with bacterial pathogens, leading to bacterial pneumonia, and they have also been associated with acute and chronic rejection, particularly in lung transplantation [180, 181]. In recent years the introduction of sensitive molecular techniques for clinical diagnosis of respiratory viruses has allowed not only early detection of these viruses [182], but also puts emphasis on other viruses like rhinovirus and metapneumovirus [183].

46.2.3.1 Respiratory Syncytial Virus

Overview

Respiratory syncytial virus (RSV) is an RNA virus that causes upper and lower respiratory tract infections, usually before 3 years of age. Reinfection is common, but, in the healthy host, it is self-limited and generally mild. Outbreaks in the community usually occur seasonally, with peaks in the late spring and autumn that last until winter [184]. There is variability of onset of infection from year to year as well as between various regions in the USA [185]. For example, in Florida, the RSV season comes earlier and lasts longer [186]. The virus may be spread in nurseries, causing severe respiratory infection in infants who have underlying medical conditions, such as bronchopulmonary dysplasia, congenital heart disease, or prematurity [187]. Viral shedding usually lasts for a week, but this period may be longer in infants who are younger than 1 month of age or in those with pneumonia [188]. Nosocomial infections often parallel outbreaks in the community.

RSV can survive drying, and it can stay viable for 6 hours on surfaces and fomites, including gloves [189]. Transmission occurs by direct contact with a person who sheds the virus in the form of droplets or from contact with contaminated hands, handkerchiefs, eating utensils, or other articles. Viral particles may be inoculated into the eyes and nasal mucosa by touching these areas with contaminated hands [189]. Therefore, nosocomial outbreaks may occur not only from patient to patient but also from caregivers or visitors having a "cold" [190, 191].

Immunocompromised patients may develop lower tract lung infection with pneumonia. RSV may infect SOT recipients [192, 193]. In recent years, cases have been reported not only in pediatric SOT populations [194] but also in adults presenting with respiratory symptoms [195]. Two liver transplant recipients younger than 15 months of age were intubated when symptoms began soon after transplantation, but they later died from RSV pneumonia [193]. This may suggest the direct inoculation of the virus in the lower respiratory tract, bypassing the upper airways. Ribavirin, administered orally or intravenously, may reduce the morbidity and mortality due to RSV, influenza B, and parainfluenza [196]. Nevertheless, its routine use has not been recommended because of possible toxicity to exposed healthcare providers (the inhalation form) and because of ongoing debate regarding its definitive beneficial effects.

There is no vaccine available for RSV prevention. Palivizumab, a humanized murine anti-RSV monoclonal antibody, can be given as a monthly IM injection beginning prior to and continuing through the RSV season (typically November to April in the northern hemisphere) for prophylaxis in infants and children at risk for severe RSV infection [197]. In a survey of pediatric solid organ transplant centers in the USA, almost 50% of responding centers use palivizumab prophylaxis [198].

Infection Prevention and Control Measures

Infection control measures should be promptly instituted to prevent nosocomial transmission. Contact precautions should be used for infants, young children, and immunosuppressed individuals. Gloves and gowns should be used when entering the room of patients with RSV, parainfluenza, or adenovirus to prevent contact with respiratory secretions. Mask and eye protection is necessary if procedures that generate vaporization of respiratory secretions are expected [2]. In outbreaks, cohorting of symptomatic patients while emphasizing hand hygiene may reduce transmission to others. Successful cohorting requires the early diagnosis of RSV when the epidemic is starting in the community. Shell vial cultures and rapid antigen detection by immunofluorescent assay (IFA) or enzyme-linked immunosorbent assay (ELISA) have greatly accelerated the diagnosis, compared with viral isolation techniques [199, 200]. More recently, more institutions have used PCR for the diagnosis of respiratory viruses, a molecular technique which is more sensitive [182, 201].

46.2.3.2 Other Respiratory Viruses: Influenza, Parainfluenza, and Adenovirus

Other respiratory viral infections that usually manifest as self-limited upper respiratory tract illness may result in potentially life-threatening lower respiratory infections in immunocompromised patients. At the University of Pittsburgh Medical Center, influenza was more prevalent among lung transplant recipients than it was among other organ recipients [202]. Secondary bacterial pneumonia occurred in 17% of the patients with influenza. Other complications occurred in three patients, including myocarditis, myositis, and bronchiolitis obliterans. Reports of transplant recipients who received the influenza vaccine but who developed influenza despite vaccination have been published [203]. This is due to the suboptimal response of transplant recipients to protein vaccines, and it raises the question of the use of antiviral chemoprophylaxis in the future. During the H1N1 April 2009 influenza A pandemic, of the reported 237 solid organ transplant recipients with H1N1, 32% developed pneumonia, 16% were admitted to ICU, and 4% died [204]. Organ recipients, their families, and the healthcare providers must realize the importance of receiving the annual inactivated influenza vaccine to reduce the risk of disease transmission. CDC, the Advisory Committee on Immunization Practices (ACIP), and the HICPAC recommended that all US healthcare workers get annual influenza vaccine [205]. The live, attenuated influenza vaccine (LAIV) administered as a nasal spray is not recommended for immunocompromised patients but may be given to close contacts of immunosuppressed individuals, though persons caring for patients in a protective environment should avoid contact with such patients for at least 7 days after receipt of LAIV [206].

Parainfluenza and adenoviruses may also cause life-threatening infection, and they may also be spread nosocomially [207, 208]. Rapid identification of these respiratory viruses, especially in pediatric wards, will help in cohorting staff and patients when an epidemic is recognized in the community [209]

46.2.4 Rotavirus and Viral Gastroenteritis

46.2.4.1 Overview

Viral gastroenteritis is usually a self-limited syndrome in the healthy host. Several viruses are associated with gastroenteritis, including rotavirus, norovirus, enteric adenovirus, caliciviruses, enteric coronavirus, and astrovirus [210]. Rotaviruses and noroviruses are the most epidemiologically significant agents of the gastroenteritis viruses, causing endemic and epidemic disease throughout the world. In particular, the rotaviruses have been associated with outbreaks in children and in developing countries where they have been associated with high mortality rates [211]. The symptoms often include vomiting, diarrhea, and dehydration. Fever may be present. Dehydration may be severe enough to

require hospitalization for intravenous fluid replacement. The incubation period ranges from 1 to 3 days, with symptoms usually lasting less than 1 week. The transmission of rotavirus occurs through the fecal–oral route, with maximum viral shedding in the stool occurring 2–5 days after the onset of diarrhea. Nosocomial infections have been associated with the insufficient use of appropriate infection control measures. Rotavirus infections represent between 20% and 40% of the cases of nosocomial diarrhea in children [212, 213]. There has been an association between rotavirus gastroenteritis and rejection of small bowel allograft but this may have been related to decrease in immunosuppressive agents due to diarrhea [214, 215]. In the USA, most infections occur in children between the ages of 6 and 24 months after maternal antibody protection wanes [216]. Infections occur more frequently between October and April. Usually, the virus produces a self-limited diarrhea; however, premature infants and transplant recipients may develop severe disease [210]. Although rotaviruses do not generally cause bloody stool, fecal occult blood loss has been reported in pediatric liver transplant recipients [217].

46.2.4.2 Healthcare-Associated Transmission

Rotaviruses can remain viable in water and on dry inanimate objects and hands for many days. An investigation in day care centers with rotavirus outbreaks demonstrated the virus by PCR on toys (39%) and environmental surfaces (21%) [217]. Rotavirus may be transmitted to the patients by aerosols. Contamination of inanimate objects occurs not only by feces but also by aerosols generated by bedpan cleaning [211]. Patient-to-patient transmission may result in mini-epidemics within the hospital [210]. Adult contacts of patients with rotavirus may exhibit subclinical illness [213]. Infection control measures should be instituted promptly whenever patients are incontinent or develop diarrhea. Standard precautions are adequate unless the patient is incontinent or diapered; contact precautions should be added in such cases. Good hand hygiene is essential, with glove and gown usage for patient contact if fecal contamination is likely. Cleaning of room surfaces with an EPA-registered hospital disinfectant is adequate for cleaning of surfaces in the patient's room [5]. The institution of infection control measures interrupted an outbreak in a pediatric oncology ward that was presumed to have occurred through contaminated toys [218]. These included contact precautions, and the daily cleaning of playroom with a dilute bleach solution.

46.2.4.3 Vaccination

The first rotavirus vaccine approved in 1998 in the USA was the Rotashield (Wyeth–Lederle Vaccines and Pediatrics), which was taken off the market 1 year later because of its association with intussusception [219]. In February 2006, a new vaccine, RotaTeq (Merck and Co.), was licensed in the USA. This is an oral live vaccine which was developed from human and bovine virus and has not been associated with intussusception [220]. It is given in three doses at 2, 4, and 6 months with completed administration by 32 weeks of age. A second vaccine Rotarix (GlaxcoSmithKline) was licensed in April 2008. It is live attenuated oral vaccine and is given to infants in two doses at 2 and 4 months infants. The two vaccines are equivalent. Although the original studies have not shown an association, post-licensure studies did demonstrate low risk of intussusception in certain populations [221], and support for use of the vaccine is universal. Between November 2007 and May 2008, delayed onset and reduced rate of rotavirus infection was observed [222], attributed to the introduction of the rotavirus vaccine. There are no data available regarding the safety and efficacy of this vaccine in immunosuppressed infants. It is believed that infants who live in the same household with immunosuppressed patients can still be vaccinated despite the small risk of transmission of the vaccine rotavirus [219].

46.3 Summary

Good infection prevention and control practices are essential for protecting highly susceptible transplant recipients. Quality management and patient safety initiatives have become driving forces in providing better patient outcomes. Insurers are interested in infection data to identify programs that have superior patient results. All of these process improvement initiatives are balanced by the evaluations of cost-effectiveness. Although some preventive measures, such as LAF, are highly effective, they may be too costly for routine use if they provide no additional benefit to the patient. While scientists are validating the use of new strategies, a renewed focus on best practices, including such basic concepts as hand hygiene, cleaning, disinfection, and preventing infections, is essential.

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Part IX
Immune Reconstitution Strategies for Prevention and Treatment of Infections

47
Vaccination of Transplant Recipients

Per Ljungman

47.1 Introduction

Over the last 40 years the numbers of solid organ (SOT) and hematopoietic stem cell transplant (HSCT) patients have increased rapidly. The most attention, until recently, has been focused on preventing infections occurring during the early phase after transplantation usually through antimicrobial chemoprophylaxis. As the results of transplantation have improved, the numbers of long-term survivors increased. Many patients remain immunosuppressed for a long time either due to the interaction between the graft and the host, i.e., GVHD in HSCT recipients or by the immunosuppressive therapy given to prevent graft rejection in SOT recipients.

Immunizations are important for two main reasons. Obviously, the most important is the need to protect the recipient against serious infections that may occur during the early or late post-transplant period. However, another reason is the public health point of view namely to avoid having individuals vulnerable to important infectious agents, for example, measles and who can serve as sources of infection in the community. Both reasons require analysis of risks and benefits for the transplant recipient.

47.2 Hematopoietic Stem Cell Transplantation

47.2.1 Allogeneic HSCT

47.2.1.1 Transfer and Persistence of Immunity in Allogeneic HSCT Recipients

After an allogeneic HSCT, the immune system of the recipient is replaced by the immune system of the donor. The immune deficiency is caused by a combination of the preparative regimen given before HSCT, GVHD, and immunosuppressive therapy given after the transplantation. Many studies have shown that immunity to some infectious agents can be transferred by the graft and be detectable in the patient early after the HSCT [1–8]. Thus, it would make sense to immunize the donor pre-donation to improve the transfer of immunity. Studies have shown that pretransplant vaccination of the donor with tetanus toxoid, diphtheria toxoid, hepatitis B virus, *Haemophilus influenzae* type B (HIB), and pneumococcal conjugate vaccine increase the probability of transfer of B-cell immunity by the stem cell graft [5, 9–11]. The clinical effects resulting from the transfer of donor immunity have been best studied for hepatitis B virus [9, 12]. Case reports suggest that transfer of immunity from an HBV immune donor can clear detectable virus from an HBV-antigen and DNA positive recipient [13, 14]. Whether this transferred immunity can prevent a primary infection is unknown. It has also been shown that transfer of donor immunity is only of a finite duration unless also the recipient is vaccinated early after transplantation [6, 9].

There are no effective and safe vaccines available for most infections that are common and serious early after allogeneic HSCT. The infections for which pre-transplant vaccinations could be considered are influenza, HIB, pneumococci, and varicella-zoster virus (VZV). Molrine et al. showed that donor immunity to pneumococci can be transferred and early post-transplant vaccination with the conjugated pneumococcal vaccine (PCV) improved the immune response [10]. In contrast, donor immunization with the 23-valent polysaccharide vaccine did not improve the recipient immunity [11]. Ambati et al. studied influenza vaccine pre-transplant with either the donor or the recipient vaccinated followed by recipient vaccination at 6 months after transplantation [15] and showed some positive effect of vaccinating the recipient but no effect of vaccinating the donor. However, it is possible that an earlier post-transplant vaccination could have been beneficial.

Another infection for which transfer of donor immunity could be of importance is CMV. There are vaccines in development that might be used in future studies.

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There are several practical problems such as the timing of donor vaccination to give optimal conditions for immunity transfer. Furthermore, the ethical aspects of donor vaccination have to be considered, for example, to vaccinate children or unrelated donors with the aim to improve transfer of a specific immunity to the recipient when no efficacy data from donor vaccination exist.

Despite that transfer of specific immunity after allogeneic HSCT does occur, the long-term presence of specific antibodies after HSCT is not only dependent on the donor's immune status but also on the recipient's immune status before HSCT [6, 16, 17]. In most patients specific B-cell immunity is usually progressively weakened over time and an increasing number of patients becomes susceptible to infections such as tetanus [3, 17], poliovirus [18–20], and measles [16, 21] during extended follow-up. The loss of protective immunity is more rapid in patients who were immunized against measles compared to those who became immune after natural measles disease (Figure 47-1) [16]. Thus, the intensity of the original antigen challenge is of importance for the duration of specific immunity after HSCT.

The T and B cell immunity post-transplant must have been at least partially reconstituted for a vaccine to induce a clinically relevant immune response. B cell counts, which are very low in the first 1–3 months after HSCT, return to normal by 3–12 months post-transplant. In patients treated with rituximab post-transplant, B cell recovery is generally delayed for at least 6 months [22]. T cell counts are low in the first 1–3 months post HSCT. Thereafter, the recovery of T cells is influenced by patient age at HSCT, T-cell depletion of the graft, and eventual chronic GVHD. The technology of allogeneic HSCT is developing rapidly with the introduction of new stem cell sources (cord blood stem cells) and new donor categories (haplo-identical donors). Data are still limited regarding vaccination responses in these subgroups of allogeneic HSCT patients and current recommendations do not differentiate between these different subgroups [23, 24].

47.2.2 Studies of Immunizations in Allogeneic HSCT Recipients

Severe infections frequently occur early after HSCT when immunizations are ineffective. It would therefore be logical to immunize candidates before HSCT. Although most studies show that adult patients with hematological malignancies respond poorly to vaccination, vaccination prior to transplant may improve immunity [25], and guidelines suggest administering inactivated vaccines prior to HSCT if there is an interval of ≥2 weeks before initiation of immunosuppressive therapy [23, 24].

A T-cell response after vaccination can be induced 2–6 months after HSCT while antibody responses usually do not develop until 6–12 months after HSCT. Vaccines based on protein antigens such as tetanus toxoid or conjugated-polysaccharide-vaccines are able to elicit immune responses earlier after HSCT than polysaccharide-based vaccines. GVHD and/or its treatment might decrease T-cell and antibody responses to vaccines although studies show limited differences in responses in patients with or without chronic GVHD [20].

Current recommendations for vaccination of HSCT recipients are shown in Table 47-1. These are based on a recent consensus document [23, 24]. It should be recognized that risk/benefit and cost/benefit ratios might be different in different countries. The local epidemiological situation for certain infections such as measles or yellow fever as shown by recent outbreaks might make vaccination with a live vaccine the preferred option despite the risk for side effects.

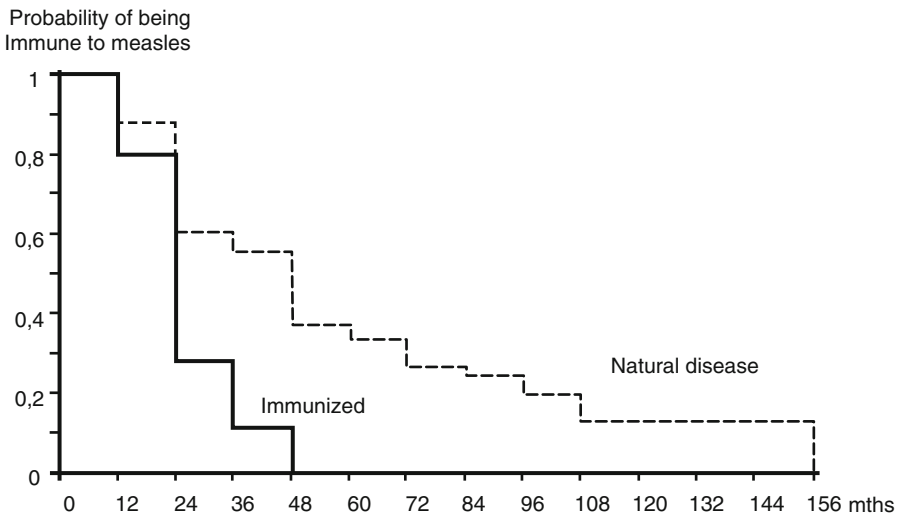

Figure 47-1. Kaplan-Meier probability for immunity in patients who had previous measles disease or who were immunized against measles. This research was originally published in Blood. Ljungman P, Lewensohn-Fuchs I, Hammarstrom V, Aschan J, Brandt L, Bolme P, et al. Long-term immunity to measles, mumps, and rubella after allogeneic bone marrow transplantation. Blood. 1994;84(2):657–63. © the American Society of Hematology.

TABLE 47-1. Vaccinations in stem cell transplant patients

Vaccine	Schedule	Comments
Pneumococcal conjugate vaccine (PCV13)	Three doses starting at 3–4 months after HSCT	A booster with either PCV13 or polysaccharide vaccine (PPV23) might be given
Haemophilus group B conjugate	3 doses starting at 3–6 months	
Meningococcal conjugate	Two doses given at 6–12 months	Follow national recommendations
Tetanus toxoid	3 doses starting at 6 months	Full dose
Diphtheria toxoid	3 doses starting at 6 months	Full dose (D)
Pertussis	3 doses to children <7 years	Full dose (P)
BCG	Not recommended	Risk/benefit ratio does not favor vaccination
Other live bacterial vaccines	Not recommended	Risk/benefit ratio does not favor vaccination
Inactivated influenza vaccine	Yearly staring at 4–6 months depending on season	Children <9 years should receive two doses. Family members and staff
Inactivated poliovirus vaccine	Three doses started at 6 months	
Hepatitis B virus	Three doses started at 6 months	The indication for HBV vaccination depends on the epidemiological situation for HBV in the country
Hepatitis A virus	Can be started at 6 months	Safe, can be considered in patients that might become exposed
MMR	1–2 doses started at 24 months	Only to patients without GVHD or ongoing immunosuppression
Varicella	Usually not indicated. Not before 24 months after HSCT	To seronegative patients without GVHD or ongoing immunosuppression
Zoster vaccine	Usually not indicated	To seronegative patients without GVHD or ongoing immunosuppression.
Other live viral vaccines	Not before 24 months after HSCT Usually not indicated	Risk/benefit ratio has to favor vaccination.

47.2.2.1 Inactivated Vaccines

Influenza Vaccine

Influenza A and B infections can be severe and life threatening in HSCT recipients and can occur several years after HSCT [26–29]. Only limited data exist in patients having received reduced intensity conditioning regimens [30] and there is almost no data in patients receiving cord blood or haplo-identical grafts. Existing data in children are also limited. In addition, with one exception, studies report the effect of the vaccine in inducing antibody responses and not protection against infection or indeed severe disease.

When vaccination should be initiated after HSCT depends in part on when an influenza outbreak is likely to occur in the patient's community. It has been shown that the time after HSCT is important for vaccine efficacy; patients vaccinated later after HSCT have better responses [31–33]. Engelhard et al. studied the antibody response to two doses of influenza virus vaccine in 35 allogeneic bone marrow transplant recipients (adults and children), who received a T-depleted transplant and vaccinated between 2 and 82 months after transplantation [34]. No patient vaccinated before 6 months from transplantation responded while more than 60% of the patients responded when vaccinated more than 2 years after HSCT. The second vaccine dose had only a marginal effect. In a study by Pauksen et al., the response rates were similar in patients vaccinated between 4 and 12 or >12 months after HSCT. Several studies were performed in HSCT recipients with the pandemic H1N1 vaccine. Issa et al. studied 82 patients vaccinated a median of 19 months after transplantation (2.5–94 months) with one dose of unadjuvanted vaccine [35]. Protective titers were found in 51% of the patients with better responses seen in patients vaccinated later after HSCT (OR 1.79/year) and poorer responses if the patient had received rituximab during the last year [35]. Engelhard et al. studied 55 patients vaccinated a median of 27 months (1–290 months) with two doses of an AS03-adjuvanted vaccine (Pandemrix, GSK). The protection rate after two doses was 48.7% and the seroconversion rate 41.9% [31]. Factors influencing seroconversion was the lymphocyte count and donor type but there was no effect of time after HSCT [31]. De Lavaillade et al. studied 26 allogeneic HSCT recipients receiving two doses of the same adjuvanted vaccine at a median time from HSCT of 39 months (6–127 months) [33]. The seroprotection rates were 45% and 73% after one and two doses, respectively, and significantly lower than in healthy controls. There was a positive effect of longer time after HSCT. Dhédin et al. reported similar immune responses after two doses of adjuvanted pH1N1 vaccine compared to natural infection [36]. No increased risk for side effects was found in any of the studies. Dhédin et al. reported, however, that four patients developed worsened chronic GVHD [36].

For inactivated influenza vaccine data regarding the effectiveness of a second dose in older children and adults have given conflicting results [31, 33, 37]. Children younger than 9 years of age who are receiving influenza vaccine for the first time require two doses administered 4 or more weeks apart. However, despite suboptimal immune responses, there might still be clinical effectiveness of vaccination. Machado et al. found that influenza vaccination performed at least 6 months after SCT had an efficacy in preventing influenza of 80% [30].

Even in cases where there is no serological response, T-cell responses may be elicited that possibly may prevent serious disease [33, 38, 39]. It is also possible that adjuvanted vaccines might improve immune responses although no controlled trial has been performed [39].

Yearly influenza vaccination is recommended to all allogeneic HSCT patients from 4 months after HSCT if there is a community outbreak and yearly thereafter [23, 24, 40]. Live attenuated influenza vaccine should not be used because the safety and efficacy of this vaccine in HSCT patients has not been established and an inactivated vaccine alternative exists. It is also recommended to immunize family members and hospital staff thereby reducing the risk for transmission of the infection to the patient [40].

Hepatitis B Virus (HBV) Vaccine

HBV infection is a major cause of morbidity in many parts of the world. One specific problem occurs when a seronegative HSCT patient is scheduled to receive a HBsAg positive marrow graft since these patients might develop severe HBV primary HBV infections after the HSCT [41]. Immunization against HBV in normal individuals usually requires three injections spread over a couple of months to ensure protective immunity. This might cause a problem when scheduling a HSCT. Furthermore, a subset of 5–15% of normal individuals will not respond to HBV immunization. No data exists regarding eventual protective efficacy of pretransplant immunization of the marrow recipient.

Immunization with HBV vaccine early after HSCT is likely to be ineffective unless the donor is immunized. The immunization of the marrow donor allows a transfer of immunity to the recipient [9, 12]. A transferred donor immunity can be long-lasting in at least 50% of the patients but revaccination of the recipient after HSCT is needed to obtain long-term immunity [42]. Vaccinated patients are significantly less likely to experience HBV reactivation compared to unvaccinated controls [43, 44]. Vaccination with HBV is recommended to allogeneic HSCT recipients from 6 to 12 months after HSCT but should be combined with antiviral prophylaxis [45].

Pneumococcal Vaccines

Fatal infections with pneumococci can occur both early and late after transplantation [46]. The risk for severe infections is increased in patients with chronic GVHD [47–49]. Protective immunity against pneumococci is mediated through antibodies directed against polysaccharides from the bacterial capsule. Two types of pneumococcal vaccines are licensed in most countries: conjugate vaccines (PCV) either containing 10 or 13 serotypes and a polysaccharide vaccine containing 23 serotypes (PPV23). PCV is more immunogenic than PPV23. However, the spectrum of protection is narrower since the vaccine contains fewer serotypes. Immunization with PPV23 can elicit antibody responses 6–12 months after HSCT in patients without GVHD but has been ineffective in eliciting adequate immune responses in patients with chronic GVHD [50–54]. In particular the specific IgG2 responses have been poor [54, 55]. The immune response was not significantly improved by two doses of pneumococcal vaccine as compared to one dose [50]. Children given pneumococcal polysaccharide vaccines had short-lived responses and low avidity [56] in contrast to adults in whom the avidity was good [57]. The functional capacity of the vaccine induced antibodies has also been studied. Parkkali et al. studied the opsonophagocytic activity against pneumococcal subtype 19 F and found that it was poor [58].

Three prospective trials demonstrate good antibody responses with the use of PCV7. Cordonnier et al. performed a randomized, controlled non-inferiority trial comparing vaccination with three doses of PCV7 given at 3 or 9 months after HSCT and there was no difference in the response rates between the two groups although patients in the early group lost immunity more rapidly especially those with chronic GVHD [59]. Molrine et al. performed a randomized study with PCV7 comparing pretransplant vaccination of the patients and their donors or no vaccination pretransplant. All patients thereafter received three doses of the vaccine at 3, 6, and 12 months after transplantation. The majority of patients (72–100% for different serotypes) developed protective antibody protection at 12 months after HSCT [10]. There was also an improved response (67% vs. 36%) to the first dose. Kumar et al. [60] conducted a randomized study comparing PPV23 versus PCV7 given to the donors before transplant and to the recipients 6 months post-transplantation. Rates of response at 12 months were low in both groups but better with PCV7. Meisel et al. immunized 53 children with 3 monthly PCV7 doses starting 6–9 months after transplantation [61]. A response (antibody titres ≥0.5 μg/ml) was noted in 74% of patients. A prospective uncontrolled trial studied the effect of four doses of the PCV13 and showed strong immune responses to the fourth dose. However, this dose resulted in an increased rate of side effects [62]. There was no beneficial effect of a subsequent dose of PPV23 but this dose was probably given too early after the fourth PCV dose to be ideal. It is also most likely necessary to give additional pneumococcal vaccine doses during follow-up of HSCT recipients to maintain immunity to pneumococci but the exact schedule needs to be defined by further studies [63].

The current recommendations are to use PCV and start vaccinating 3–6 months after HSCT. In patients with chronic GVHD a fourth dose is recommended while in patients without chronic GVHD, a dose of PPV23 is recommended to broaden the immune response [23, 24].

47.2.2.2 H. influenzae Type B Vaccine

H. influenzae can also cause severe infections in allogeneic HSCT recipients although fatal infections seem to be rare. Vaccination with conjugated HIB vaccine can elicit protective immune responses [50, 51, 53]. However, the time after HSCT is important since it was reported that early vaccination gave

poorer responses in transplanted children [64]. Immunization with the HIB vaccine is indicated to all allogeneic HSCT recipients [45, 65, 66].

Tetanus Toxoid, Diphtheria Toxoid, and Poliovirus Vaccine

Most HSCT patients will lose immunity to tetanus toxoid, diphtheria toxoid, and poliovirus during extended follow-up. Tetanus is a rare but life-threatening disease preventable by immunization. Sporadic cases of diphtheria do occur in many countries. Although the efforts to eradicate polio have been successful in many parts of the world, small outbreaks still occur in non-immune populations. Thus, there are good reasons for having an immunization strategy against these infections in HSCT recipients.

Several studies of immunization with these vaccines have been published [17–20, 67]. A new primary schedule with repeated doses of these vaccines is needed to obtain stable protective immunity [17–19]. Good and lasting immune responses can be obtained when immunizations are given at 6 months after HSCT [20, 67]. Gerritzen et al. immunized children before HSCT followed by revaccination already at 6 weeks after HSCT. Thirty percent of the patients responded to early immunization [68].

The inactivated poliovirus vaccine should be used to prevent a vaccine induced paralytic disease. It is also important that the inactivated vaccine is used in family members to HSCT patients and in hospital staff caring for these patients since transfer of the live vaccine virus from an immune competent to an immunosuppressed individual has been reported.

The vaccination of allogeneic HSCT recipients with three doses of tetanus toxoid, diphtheria toxoid, and inactivated poliovirus vaccine starting at 6–12 months after HSCT is recommended to all allogeneic HSCT recipients [45, 65, 66].

Pertussis Vaccine

HSCT recipients might be vulnerable to complications from pertussis due to pulmonary damage from chemotherapy and/or TBI, although the documentation for severe infections is limited [69, 70] even in the absence of chronic GVHD. In young children, it is logical to vaccinate against pertussis together with diphtheria, and tetanus since many available vaccines include the pertussis component (DTaP) some with added HIB and IPV components.

The immune response to vaccines containing low amounts of pertussis antigen (Tdap) has been shown to be poor [71]. In view of this, vaccines containing higher pertussis content are recommended. Whether combination vaccines have the same effect in adults as in young children have not been studied.

Other Killed Vaccines

Vaccination with a tetravalent meningococcal polysaccharide vaccine can elicit good responses in HSCT recipients both against serogroup A and C [72]. Mahler et al. studied the tetravalent conjugated meningococcal vaccine in 46 patients, mainly children, at a median of >2 years after HSCT [73]. The response rate to the different serotypes varied between 30% and 52%. Seven of 46 patients (15% responded to all serotypes and 35% did not respond to any serotype). Sixteen patients got a second dose and 50% responded against all serotypes.

Current recommendation state that HSCT patients should receive meningococcal vaccine if recommended to the general population in the country where the patient resides [23, 24, 40].

There is no data with the human papillomavirus (HPV) vaccine but studies are needed since papillomavirus-induced tumors are more common after HSCT than in a normal population.

Hepatitis A is a severe infection in many parts of the world and the vaccine is inactivated. Thus, vaccination could be indicated. However, limited data is available in HSCT recipients.

47.2.2.3 Live Vaccines

Measles, Mumps, and Rubella (MMR) Vaccine

Measles is still an important infection in many parts of the world. Until recently, the good vaccination coverage in the general population in Europe and USA reduced the risk for outbreaks and thereby the risk for transfer to HSCT recipients. However, during the last decade the vaccination rates of children in many countries decreased and many outbreaks have been reported [74, 75]. Many patients will become seronegative to measles during an extended follow-up especially if previously vaccinated [16, 21]. This is important since occasional severe and also fatal measles infections have been reported in HSCT recipients [76, 77]. During a large outbreak in Brazil, one of eight patients with measles developed interstitial pneumonia but all survived [21].

The available measles vaccines are live, attenuated and usually combined with rubella, and mumps vaccines (MMR). These vaccines are not recommended for use in immunocompromised patients since serious side effects are possible. Immunization can only be considered in allogeneic HSCT patients without chronic GVHD or ongoing immunosuppression. Data indicates that measles vaccine can be given safely to such patients at 2 years after HSCT [78]. During an epidemic in Brazil, patients were immunized at 1 year after HSCT and no severe side effect was observed [79]. The reported effect of vaccinations varies between different studies with a higher response rate in adults than in children [78, 80–82]. A second dose might therefore be needed in children. Earlier vaccination can be contemplated if the epidemiological situation in the community indicates that there is a significant risk for measles. In such situations, it is likely that the risk/benefit ratio will favor early vaccination.

The risk for severe rubella infection after allogeneic HSCT is likely to be low. However, with more patients undergoing transplantation after reduced intensity conditioning regimens, the pregnancy potential for patients is likely to increase.

The risk for severe mumps disease seems to be very low after HSCT. The indication for mumps vaccination is therefore weak. However, most vaccines are combination vaccines including measles, mumps, and rubella (MMR) and single measles vaccine is difficult to find in most countries. Current recommendations are therefore that MMR vaccine is used but it should not be given earlier than 2 years after HSCT and only to patients not having chronic GVHD or ongoing immunosuppression [45, 65].

Varicella-Zoster Virus (VZV) Vaccine

Primary VZV infections can be very severe early after allogeneic transplantation. The existing vaccine is live and attenuated and shall therefore not be used early post-transplantation. A seronegative patient should, if possible, be immunized before transplantation if enough time can elapse from the vaccination to the transplant procedure. There is no data allowing assessment of the minimum interval that is needed between immunization and transplantation but recent recommendations state an interval of at least 4 weeks [23, 24]. This strategy has not been formally studied although it is likely that children with acute leukemia, who have been immunized with the varicella vaccine, have subsequently undergone allogeneic HSCT. Vaccination of seronegative family members to allogeneic SCT recipients is also recommended [83]. A few uncontrolled studies have been performed and reported that varicella vaccination is safe and can result in seroconversion if performed >2 years after HSCT in patients without chronic GVHD, or ongoing immunosuppression [84–86].

A high proportion of HSCT patients develop herpes zoster that occasionally becomes severe. Redman et al. used heat-inactivated varicella vaccine and showed no reduction in the risk of developing herpes zoster but a reduced severity of the herpes zoster in the immunized group [87]. Issa et al. gave one dose of zoster vaccine to 58 allogeneic HSCT recipients and did not note any significant side effects [88]. Inactivated vaccines are currently in late clinical development. The immune response in allogeneic HSCT recipients was poor in a phase II study with a heat-treated vaccine [89]. A recent study of a subunit vaccine showed promising results in healthy elderly and should be evaluated in HSCT recipients as well [90]. Due to the proven efficacy and safety of acyclovir prophylaxis [91], vaccination with the live zoster vaccine is not recommended during at least the first years after HSCT.

Other Live Vaccines

Other live vaccines are typhoid, BCG, and yellow fever. Yellow fever is a life threatening infection primarily occurring in Central- and South America and southern and central Africa. The vaccine is live and attenuated. Rio et al. have presented three patients who were immunized at 5 years after BMT without severe side effects [92] and this experience has since then been expanded to 25 patients without any serious side effects (B Rio, personal communication). Immunization could be considered in patients who must visit areas where yellow fever is endemic. It seems likely that the same limitations are indicated as for other live virus vaccines.

Both live and inactivated vaccines against *S. typhi* exist. Therefore, use of the live vaccine should be avoided. BCG vaccine can cause severe infections in patients with depressed T-cell function and is not recommended in HSCT recipients.

47.2.2.4 Long-Term Follow-Up After Vaccinations

Limited data is available regarding long-term persistence of immunity after vaccination of HSCT recipients. Figure 47-2 shows own unpublished data for immunity at 4–12 years after vaccination against poliovirus type 1 with three doses of inactivated vaccine at 12, 13, and 18 months after allogeneic HSCT.

Cordonnier et al. studied antibody levels at 10 years after vaccination in patients previously participating in a randomized study of PCV7 followed by PPV23 and showed no significant decrease in antibody levels compared to 24 months after HSCT although with significant variability between different subtypes [63].

47.2.3 Autologous Bone Marrow Transplantation

47.2.3.1 Persistence of Immunity in Autologous HSCT Recipients

In autologous HSCT recipients the immune system is depressed by high doses of chemo- and radiotherapy but there is no immunological disparity between the graft and

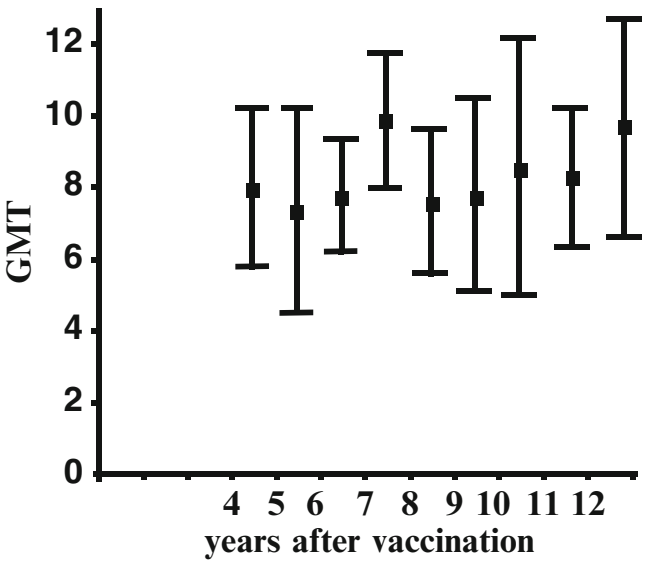

FIGURE 47-2. Geometric mean neutralization titers (± SE) against poliovirus type 1 in allogeneic HSCT patients during long-term follow-up.

the patients. The immune regeneration is, in most patients, quicker than after allogeneic HSCT and even more so after peripheral blood stem cell transplantation. Early severe infections caused by pneumococci and influenza have been reported [28, 46]. Less is known about late immune deficiency after autologous compared to allogeneic HSCT. Nordöy et al. reported that patients who had undergone autologous HSCT for lymphoma had persistent abnormalities in lymphocyte subsets for several years after the transplantation [93]. Previous treatment especially with anti-B-cell antibodies will negatively influence the immune reconstitution and responses to vaccination after autologous HSCT.

Several studies have shown that also autologous HSCT recipients will lose protective immunity to tetanus, poliovirus, and measles during extended follow-up [19, 94–97]. Similar to allogeneic HSCT recipients, poor responses to immunization are elicited if immunizations are given early after transplantation [32, 34]. One option in autologous HSCT recipients could be to immunize the patient either before stem cell harvest or just before the transplantation. Immunization against HBV elicited protective immune response when one dose was given before and one dose early after autologous HSCT [98]. However, the seroconversion was only transient in one-third of the responding patients. Immunization with conjugated *H. influenzae* type B vaccine and tetanus toxoid before marrow harvest followed by immunization at 3, 6, 12, and 24 months after autologous HSCT improved the antibody titers to both antigens already at 3 months after the transplantation [99].

47.2.4 Immunization of Autologous HSCT Recipients

Fewer studies of immunization have been performed in autologous than in allogeneic HSCT recipients. Most published recommendations do not differentiate between allogeneic and autologous HSCT recipients. Therefore, if no specific knowledge exists regarding autologous HSCT recipients, the recommendations are based on those given for allogeneic HSCT recipients.

Influenza The response to vaccination is likely to be suboptimal early after transplantation [32, 34]. In one study, no patient responded if the immunization was performed earlier than 6 months after autologous HSCT [34] and in a study that added GM-CSF to influenza vaccination, an antibody response was elicited in less than half of the patients vaccinated during the first year after HSCT [32]. It has been shown that rituximab blocks the immune response to influenza vaccine for at least 6 months after HSCT and probably longer in many patients [100–102]. Despite these limitations influenza virus immunization is recommended from 6 months after autologous stem transplantation and yearly thereafter [45].

Pneumococcal Vaccines Hammarström et al. showed that the majority of a group of autologous HSCT patients transplanted for various diagnose retained normal antibody levels to pneumococci [54]. In contrast Nordöy et al. showed in a group of lymphoma patients that the patients had lower antibody levels compared to controls already before HSCT [97]. However, there was no significant further loss of immunity after the transplant. Autologous HSCT patients are less prone than allogeneic patients to develop severe pneumococcal infections. Most infections occur early after the transplantation when the response to immunization is poor [46]. Vaccination before stem cell harvest with PCV7 resulted in significantly higher antibody levels at all studied time points up to 12 months after HSCT. However, there is no advantage vaccinating the patients before the stem cell harvest with PPV23 [11]. The response to a single dose of pneumococcal vaccination is poor regardless of the stem cell source [50, 103] and remains decreased compared to controls for several years after transplantation for lymphoma [97]. It is likely that treatment with rituximab will negatively influence the response to pneumococcal vaccination but it has not been systematically studied. Vaccination with the PCV7 has been shown to be effective and induced protective antibody levels in >60% of autologous HSCT patients [104]. Vaccination with PCV13 is recommended starting at 3–6 months after autologous HSCT [23, 24].

HIB Vaccine Immunizations with two doses of the conjugated HIB vaccine induced a protective immune response in 80% of HSCT recipients [50]. The immune response might be improved by immunizing the patient before the stem cell harvest. Immunization with a conjugated HIB vaccine before the harvest followed by immunization at 3, 6, 12, and 24 months after autologous HSCT improved the antibody titers already after the dose given at 3 months after the transplantation [99].

Tetanus Toxoid, Diphtheria Toxoid and Poliovirus Vaccine Autologous HSCT recipients have an increased risk compared to the normal population to lose protective immunity to poliovirus [95, 97], diphtheria [97], and tetanus [96]. Reimmunization with repeated doses of inactivated poliovirus vaccine, diphtheria toxoid, and tetanus toxoid effectively restores protective immunity in autologous HSCT recipients [19, 95, 97, 103, 105]. There was no difference in response in autologous HSCT or peripheral blood stem cell graft recipients [103]. Vaccination with reduced dose pertussis vaccine (Tdap) failed to induce immune responses in most autologous HSCT recipients [106]. Immunization with tetanus toxoid, diphtheria toxoid, and inactivated poliovirus is recommended after autologous HSCT [23, 24].

47.2.4.1 Live Attenuated Vaccines

Measles, Mumps, and Rubella Vaccine

Children previously immunized to measles before autologous HSCT frequently become seronegative during follow-up, but adults who before autologous HSCT experienced natural measles disease usually remain immune to measles during follow-up after transplant [94]. The risk for side effects after immunization seems to be low [94]. Earlier vaccination could be considered in high-risk epidemiological situations. Due to the lower risk to lose specific immunity in adults, determination of the antibody level and vaccination only of seronegative patients could be considered [65]. No data exists regarding efficacy and safety of mumps and rubella vaccines after autologous HSCT but are likely to be similar to the situation for measles vaccine. MMR vaccination is recommended for children and seronegative autologous patients not earlier than 24 months after HSCT [23, 24]. Two doses are recommended in children <9 years old.

Varicella-Zoster Virus Vaccine

No studies have been done with the varicella vaccine specifically in seronegative autologous HSCT patients. A small study showed seroconversion and no severe side effects in seronegative allogeneic and autologous HSCT recipients [84]. Sasadeusz et al. gave one or two doses of the live varicella vaccine 4.5–6 months after HSCT. The immune responses were rather poor but no severe side effects occurred [107]. Based on lack of information, varicella vaccination is not recommended during the first 2 years after autologous HSCT [23, 24, 45].

The only commercially available zoster vaccine is live and attenuated. Issa et al. vaccinated 52 autologous HSCT recipients around 2 years after transplantation and did not note any significant side effects [88]. There is, however, one death reported with the vaccine strain 4 years after autologous HSCT in patient with untreated NHL relapse [108]. The safety of live varicella or varicella-zoster vaccines after autologous HSCT must be balanced against other options such as acyclovir/valacyclovir prophylaxis.

Another possibility could be the use of inactivated vaccines after autologous HSCT. Redman et al. gave three doses of a heat-inactivated vaccine to a mixed group of stem cell transplant patients and was able to show a reduced severity but no reduction of the frequency of herpes zoster [87]. Hata et al. have published results of a randomized study in lymphoma patients, who had undergone an autologous transplant [109]. Four doses of vaccine were given, one before and three after HSCT. The risk for zoster was significantly reduced in the vaccinated group (13%) compared to the non-vaccinated group (33%; $p=0.01$). Furthermore, it was shown that vaccination improved the specific CD4+ response to VZV. There are now two different vaccines in development; one inactivated and one subunit vaccine. Stadtmauer et al. reported on a phase I/II placebo-controlled study in which 121 adult patients were vaccinated with three doses of the adjuvanted subunit varicella vaccine [110]. Patients receiving the vaccine had improved CD4+ and antibody responses compared to those given placebo. Mullane et al. studied four doses of the heat-inactivated vaccine. The vaccine was able to induce both antibody and cell-mediated immune responses [89]. Phase III studies are ongoing.

47.3 Solid Organ Transplant Recipients

The need for immunization SOT recipients arises from three components each causing a suppression of the immune system: the immunosuppressive activity of the underlying disease, for example, chronic renal failure, rejection of the organ graft, and the immunosuppressive therapy given after the transplantation. If possible, vaccinations should be given to candidates according to age appropriate schedules when the likelihood of an immune response is higher than after the SOT [23, 24]. The optimal time to begin vaccine administration after transplant is not defined. The immunosuppressive therapy is often most intense during first couple of months and might influence the effect of vaccination. However, this has not been well studied. A summary of the current recommendations is shown in Table 47-2.

47.3.1 Immunizations Given Before Transplantation

47.3.1.1 Hepatitis B Virus

HBV can be transmitted either by a hepatitis B antigen positive organ graft or through blood transfusions. HBV vaccination is recommended in HBV negative patients before SOT. However, the efficacy of HBV vaccine is lower in patients on hemodialysis [111, 112] and in patients with end-stage liver disease compared to healthy controls [113, 114]. In contrast, HBV vaccine elicited an immune response in 73% of children with biliary atresia awaiting liver transplantation [115]. Different schedules have been used attempting to improve the response to vaccinations including accelerated dose schedules and double strength dosages [116–119]. The seroconversion rates in the different studies varied from 31% to 62%. Factors associated with better responses were young age [116], a milder grade of liver disease [116], and specific HLA-types [116]. Besides poor responses to vaccination, the antibody levels decreased rapidly after liver transplantation so that up to 35% of the patients who had seroconverted became seronegative [116, 117]. Despite the less than optimal responses, pretransplant immunization is recommended [120].

TABLE 47-2. Recommendations for immunizations in solid organ transplant recipients

Vaccine	Recommendation before transplantation	Recommendation after transplantation	Comments
Killed vaccines			
Haemophilus group B conjugate	Yes	Yes	Age appropriate
Hepatitis A virus	Yes	Depending on serostatus	Liver transplant recipients
Influenza (inactivated)	Yes	Yes	
Hepatitis B virus	Yes	Depending on serostatus	Seronegative recipients
Pneumococcal conjugate	Yes	Yes	Age appropriate
Pneumococcal polysaccaride	Yes	Yes	Age appropriate
Meningococcal conjugate	Yes	Yes	Age appropriate
Poliovirus (inactivated)	Yes	Yes	Age appropriate
Papilloma virus	Yes	Yes	Age appropriate
Tetanus, diphtheria, and pertussis	Yes	Yes	Age appropriate
Live vaccines			
MMR	Yes	Not recommended	Age appropriate
Varicella vaccine	Yes	Not recommended	Before transplantation in seronegative patients
Zoster vaccine	Possibly; age appropriate	Not recommended	
Other live vaccines (rotavirus, yellow fever)	Possibly	Not recommended	

Pre-transplant vaccination combined with lamivudine therapy has been shown to be effective to prevent primary HBV infection in patients receiving liver grafts from either anti-HBC- or anti-HBc+ donors. Two of 60 patients (one from each donor cohort) developed a primary HBV infection during a median follow-up of almost 5 years [121]. Another possible indication for HBV vaccination would be to prevent reinfection of a liver allograft in HBV positive patients. However, this strategy has been ineffective [122].

47.3.1.2 Hepatitis A Virus

Hepatitis A virus (HAV) can cause decompensation and death in patients with chronic liver disease. Therefore, vaccination can be an important protective strategy. Patients with chronic liver or renal disease awaiting transplantation are able to respond to An HAV vaccination although the results are not as good as in normal individuals [123].

47.3.1.3 Varicella-Zoster Virus

Varicella-zoster virus can cause severe and potentially fatal disease in patients after organ transplantation. It is important to consider the vaccination of seronegative patients awaiting organ transplantation. Varicella vaccine given to uremic children awaiting renal transplantation was shown to be safe and reduced the post-transplant risk for varicella in pre-vaccination seronegative patients [124]. A follow-up study showed that the protection was long-lasting with 42% of the patients still having antibodies at more than 10 years after immunization. Furthermore, the risk for varicella was lower and the disease was significantly less severe in immunized than in non-immunized patients [125]. Similar results were seen in a study of children with chronic liver disease awaiting transplantation with a vaccination efficacy of 100% and no severe vaccine associated side effects [126].

47.3.1.4 Tetanus and Diphtheria Toxoid

Impaired vaccination responses have been reported to tetanus and diphtheria toxoid in dialysis patients. One year and five years after vaccination 35% and 32%, respectively, of patients were protected against diphtheria [127]. 65% of patients were protected against tetanus at 12 months after transplantation [127]. Children should if possible complete their primary vaccination schedule before transplantation. Boosters (Tetanus-reduced diphtheria; Td) can be considered to adults and older children.

47.3.1.5 Pertussis Vaccine

The incidence of pertussis is increasing in many countries and acellular pertussis vaccine is included in the primary vaccination schedule together with diphtheria and tetanus in many countries (DTaP). Although no data exists in solid organ transplant candidates, it is recommended that the primary DTaP schedule should be completed before transplantation if necessary by an accelerated schedule. A dose of tetanus-reduced diphtheria-acellular pertussis vaccine (Tdap) could be considered in adults and older children instead of only Td although no efficacy data exist for the pertussis component.

47.3.1.6 Pneumococcal and HIB Vaccines

Immunizations against *S. pneumoniae* and *H. influenzae* is recommended for children candidates for a SOT [128, 129]. For children below the age of 2 years, the conjugated (PCV7)

vaccine is recommended and if necessary in an accelerated fashion [130]. In older children, an additional dose of PPV23 to broaden the immune response can be considered although there is currently no data with such a strategy [130].

47.3.1.7 Measles-Mumps-Rubella Vaccines

MMR vaccinations have been given to infants awaiting renal transplantation with good responses to vaccination [131]. 88% of the patients developed immunity to all three components of the vaccine. Analyzing the components separately, 89% developed immunity to measles, 88% to mumps, and 100% to rubella.

47.3.2 Immunization Given After Transplantation

47.3.2.1 Killed Vaccines

Hepatitis B Virus Vaccine

The efficacy of HBV vaccination was reported to be low after solid organ transplantation with response rates between 5 and 15% [132, 133]. It was, however, reported that pediatric liver transplant patients can achieve good vaccination responses (70% seroconversion; [134]). The type of immunosuppression influences the vaccination response in that patient receiving triple immunosuppression (cyclosporine, corticosteroids, and azathioprine) responded less well than patients receiving cyclosporine only [134]. HBV vaccinations as protection against reactivation in patient transplanted for HBV cirrhosis have been used in two studies giving different results. Sanches-Fueyo et al. reported good results with this strategy using double dose of HBV vaccine [135] while Angelico et al. reported poor results with a similar strategy [136]. Two studies with vaccines including experimental adjuvants have given promising results with response rates of 50–80% in liver transplant recipients transplanted for HBV-related disorders [137, 138]. A booster with conventional vaccine might improve long-term retainment of immunity [139].

Hepatitis A Virus

Vaccination results after liver or renal transplantation are poorer compared to healthy individuals [140, 141] and antibody levels decrease more rapidly [141]. In a study including kidney transplant recipients in South Korea, 52 patients received two doses of HAV vaccine and the seroconversion rate was only 27% with lower serum creatinine and higher hemoglobin being positive predictors for response [142].

Influenza

Influenza can cause severe infections in SOT patients [26, 27] as shown by the experience during the 2009 H1N1 pandemic [143]. Results of immunization vary with the age of the patients. Adults have poorer antibody responses to influenza vaccinations than immune competent controls after renal transplantation [144, 145], liver transplantation [146, 147], and heart transplantation [148, 149]. In contrast, studies have shown normal responses to influenza immunization in children after renal [150, 151] [151, 152] and liver transplantation [153]. The type of immunosuppression given post-transplant influences the response. In lung transplant recipients, mycophenolate mofetil was associated with a poorer and sirolimus with a better vaccination response [154]. It has been suggested that intradermal vaccination can improve the immune response in renal transplant patients [155] but a randomized study did not find an improved effect except in subgroups [156]. Despite that systematic reviews have shown that SOT patients respond more poorly to influenza vaccine than healthy controls [157, 158], vaccinated transplant patients had a lower risk for influenza like illness and laboratory-verified influenza compared non-vaccinated controls [157]. Furthermore, Magnani et al. reported that vaccination of heart transplant recipients can reduce the proportion of patients developing influenza-like symptoms [159].

Several studies have shown no increased risk for graft rejection after influenza vaccination [135, 146, 148, 149, 159–163]. A large cohort study of >50,000 renal transplant recipients instead showed a reduced risk for allograft loss and death during the first year after transplantation in patients receiving influenza vaccine [164]. Although a possible immunizing effect cannot be entirely excluded, the benefits with influenza vaccination are far greater than the possible risks [165].

Annual inactivated influenza vaccine is therefore recommended to SOT recipients. Influenza vaccination is also recommended also to transplant candidates. No data exist with the live attenuated vaccine and it should therefore not be used [23, 24, 165].

Pneumococci and HIB

The HIB vaccine was reported being similarly effective in renal transplant recipients as in controls [166]. There have been conflicting reports regarding the efficacy of the PPV23 after transplantation. In some studies it was reported to be similar to healthy controls after liver transplantation [167], heart transplantation [148, 167], and renal transplantation [168] while in other studies, the antibody responses were reported being suppressed after heart transplantation [169] and liver transplantation [170]. Furthermore, it was reported that antibody levels declined faster than in controls [170]. Kumar et al. compared PCV7 and PPV23 in a double-blind, randomized study in renal transplant recipients. There was a trend to short term better responses after vaccination with PCV7 but at 3 years after vaccination, there was no difference and the titers had decreased significantly and similarly between the two vaccine groups [171, 172]. The same group reported no improvement by giving PCV7 followed by PPV23 (prime-boost strategy compared to PPV23 alone) [173].

Tetanus and Diphtheria Toxoid

In children, who had undergone renal transplantation, immunity to diphtheria was found in 38% and to tetanus in 90% [174]. Thus, immunity to tetanus seemed to be more d than immunity to diphtheria. A booster vaccination gave protective antibody levels to diphtheria in 95% if the patients but at 12 months only 76% remained protected [174]. All patients became protected against tetanus with antibody levels comparable to those reached in normal children. Boosters with tetanus and diphtheria (Td) are recommended following SOT.

Papillomavirus Vaccine

SOT patients are at higher risk for human papillomavirus-related genital warts, cervical cancer, and other anogenital malignancies. A small study in adolescent kidney and liver transplant recipients showed a 100% response rate to vaccination with the quadrivalent HPV vaccine [175]. Indications for vaccination against papillomavirus should be the same as for the general population but due to an increased rate of warts, the quadrivalent vaccine might be preferable in SOT candidates and recipients [23, 24].

Other Vaccines

No studies have been performed in solid organ transplant patients with meningococcal conjugate vaccine. However, the risks with these vaccines are negligible and vaccination could be considered as appropriate for age and the epidemiological situation.

In a phase II study, a gB based CMV vaccine given to seronegative patients receiving grafts from CMV seropositive donors given before and twice after liver and renal transplant patients was able to induce specific antibodies and was furthermore associated with fewer days of CMV viremia and fewer days of ganciclovir therapy [176]. A study performed in heart transplant patients reported lower vaccine response to tick-borne encephalitis vaccine compared to healthy controls [177].

47.3.2.2 Live Vaccines

Immunization with live vaccines is not recommended following SOT due to the risk for vaccine associated complications. Varicella vaccine has, however, been given to small groups of seronegative transplant recipients after transplantation and existing data suggest safety and reasonable effectiveness. A small study of varicella vaccination in renal transplanted children has been published showing good serologic responses and no severe side effects [178]. Weinberg et al. vaccinated 16 seronegative liver and intestine transplanted children 8.5 months to 5.5 years after transplantation [179]. Five patients developed mild side effects 3 of whom were given acyclovir. 87% developed both humoral and cell-mediated immune responses and no patient has during follow-up developed varicella despite five reported exposures. Similarly, Khan et al. reported seroconversion in 20/31 of varicella vaccinated pediatric liver transplant recipients 7 of whom required repeated doses [180]. In a study of 16 pediatric liver and small bowel transplant recipients, 87% of the patients developed both humoral and cell-mediated immunity [179]. Three of the 16 patients were given acyclovir for vaccine induced rash. However, there are also reports of significant varicella virus disease after varicella vaccination to SOT recipients [181, 182] and further studies are needed to assess both efficacy and safety of this approach.

A small series of liver transplant patients immunized with measles vaccine showed a rather poor response (7 of 18 immunized seroconverted) but no vaccine associated side effects were found [183]. Khan et al. retrospectively analyzed 26 liver transplanted children receiving MMR vaccine and 19 seroconverted although 18 needed repeated doses [180]. Only minor side effects were seen. Additional studies are needed to assess safety especially of MMR vaccination. Two case reports have been published of measles-associated encephalopathy in children with renal grafts [184].

There is no data regarding vaccination with other live vaccines such as rotavirus, yellow fever, or BCG either to candidates or after transplantation. However, infants awaiting transplantation can be vaccinated with live rotavirus vaccine according to national guidelines. This vaccine should not be administered after SOT.

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48
Adoptive Immunotherapy for Infection Control Using Antigen-Specific Donor-Derived T Cells After Transplantation

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48.1 Potential of Adoptive Immunotherapy for Prevention or Treatment of Infections After Allografting

From an evolutionary perspective it was the immune systems major role to distinguish between "friend and foe" leaving the role of the foe largely to viral, bacterial, parasitic, or other microbiological invaders. In contrast, today's understanding of cellular immunotherapy puts a clear focus on the treatment of malignancies. This may be partly due to the fact that its most successful offspring, the transplantation of an allogeneic immune system has been providing cure to patients with otherwise fatal hematological and lymphatic diseases for several decades by now. However, from a broader perspective immunotherapy should be regarded as the transfer of cellular compartments of an autologous, allogeneic or xenogeneic immune system offering the opportunity to provide long-lasting beneficial effects to the recipient. These desired effects may include anti-malignant or anti-infectious properties or both and may also restore the host's immunological integrity in cases of imbalance such as immunodeficiency, GvHD, or autoimmune disorder. However, a primary function of the cellular immune system is to afford protection against pathogens such as viruses compromising the host's integrity. Therefore, allogeneic hematopoietic stem cell transplant (HSCT) and solid organ transplant (SOT) recipients who receive immunosuppressive therapy, which interferes with cellular immune functions, are prone to severe and potentially fatal infections, so efforts to restore protective immune responses by cellular immunotherapy are beneficial. Due to the conceptual attraction of immunotherapeutic approaches to infections in transplant recipients and the success of immunotherapy in experimental animal models and based on novel cell selection technologies, efforts to translate the basic principles that have been established in animal model studies to the clinical treatment of infections has made major progress. The objective of this chapter is to review the principles underlying cellular immunotherapy and the increasing number of strategies to apply adoptive immunotherapy for infections that are prevalent in immunocompromised transplant recipients.

Transplant recipients are vulnerable to progressive infection following the acquisition of a primary viral infection and from the reactivation of viruses that have previously established a latent or persistent infection in the host. Acute infection with seasonal respiratory viruses, such as influenza virus, respiratory syncytial virus (RSV), and parainfluenza virus, is a cause of serious infection in immunocompromised hosts during periods when infections with these viruses are prevalent in the community [1–5]. Infection of transplant recipients with such respiratory viruses can be self-limiting, as observed in immunocompetent hosts, but it often progresses to severe pneumonitis and death [1–3, 5]. Studies in murine models suggest that both B-cell and T-cell immunity are important for preventing and resolving these infections, but the unpredictable occurrence of these outbreaks and the antigenic diversity of the causative viruses make them less attractive as candidates for studies of cellular immunotherapy to restore potentially protective immune responses [6, 7].

Historically, a more prevalent problem for transplant recipients has been the acquisition of primary infections with cytomegalovirus (CMV) and Epstein–Barr virus (EBV), both of which could be transmitted to previously unexposed recipients by blood products and/or the hematopoietic stem cells or a solid organ transplant. If the transplant donor and recipient are both seronegative for CMV, the use of blood products from CMV-seronegative donor for transfusion can prevent primary infection of the recipient [8]. However, CMV-seronegative or EBV-seronegative HSCT or SOT recipient receiving hematopoietic stem cells or an organ from a CMV-seropositive or EBV-seropositive donor is at risk for the development of progressive disease caused by these viruses.

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The reactivation of endogenous latent viruses of the herpes group, including herpes simplex virus (HSV), varicella-zoster virus (VZV), CMV, EBV, human herpesvirus 6 (HHV-6), and potentially HHV-8, also results in significant morbidity and mortality in immunocompromised transplant recipients [8–11]. The administration of ganciclovir for CMV infection either prophylactically to prevent reactivation or after reactivation when it is detected by culture, antigenemia, or polymerase chain reaction (PCR) methodology is highly effective for preventing diseases in both allogeneic HSCT and SOT recipients, but it frequently causes neutropenia, especially after HSCT [12–17]. Studies in HSCT recipients have also demonstrated that the use of ganciclovir early after transplantation to prevent CMV disease may be associated with a delay in the reconstitution of CMV-specific T-cell immunity and with an increased incidence of late-onset CMV disease (disease occurring more than 100 days after transplantation) [18–22]. New antiviral agents (brincidofovir (CMX001) and letermovir) to be used for prophylaxis might have an impact on the incidence of CMV infection and early and late disease posttransplant [23–30]. Anti-CD20 antibody therapy has been successful in some patients with EBV-induced lymphoproliferative disorder (EBV-LPD) [31], but the emergence of CD20 negative B cells and PTLD in a subset of patients treated with anti-CD20 suggests that additional options to treat EBV-LPD are needed. Thus, although antiviral drugs constitute an important advance for the prevention or resolution of herpes viral infections, especially those occurring in the early posttransplantation period, the long-term control of persistent herpesviruses may require the host to mount and maintain an adequate immune response to the respective pathogen.

48.2 Effector Cell Populations and Control of Viral Infection

The development of cellular immunotherapy for individual viral infections should be predicated on an understanding of the nature of the host response that provides protective immunity in immunocompetent hosts. After acute infection with a pathogen, the healthy host mounts a multifaceted and coordinated immune response. The cellular components of this response can be broadly divided into the following two categories: (1) effector cells that are not antigen specific, such as natural killer (NK) cells and macrophages, which may be important in the initial containment of infection and (2) effector cells that express surface receptors that convey a high degree of specificity for antigens expressed by the virus, such as αβ+ T cells, γδ+ T cells, and antibody-producing B cells.

NK cells recognize and lyse target cells expressing low levels of class I major histocompatibility complex (MHC) molecules, and they might be expected to participate in the host response against viruses, such as CMV, HSV, and adenovirus, which downregulate the expression of class I MHC molecules in the infected cell [32–35]. NK cells exhibit antiviral activity in vivo, as studies in mice and in humans with selective NK deficiency have demonstrated, and they may be important during the early phase of infection prior to the development of virus-specific T cells [36, 37]. However, viruses such as CMV express genes that inhibit NK cell activation and lysis of the infected cells, potentially limiting the efficacy of NK cells in established infection [38]. In some reports, the administration of activated NK cells in patients with malignant disease has been associated with significant toxicity [39]; in others, NK cell transfer was extremely well tolerated even in the setting of haploidentical stem cell transplant (SCT) [4–42].

γδ+ T cells that recognize virus-infected cells have been identified, and these could be of benefit in adoptive immunotherapy [43, 44]. There is an increasing evidence that γδ T cells have potent innate antitumor and anti-infective activity [45]. Long-lasting expansion of Vdelta2(−)γδ T cells is a hallmark of CMV infection in kidney transplant recipients. γδ Tcell clones have been derived from several transplanted patients, and Vdelta1(−), Vdelta3(+), and Vdelta5(+) T-cell clones expressing diverse Vgamma chains, but not control Vgamma9Vdelta2(+) T clones, displayed strong reactivity against CMV-infected cells, as shown by their production of tumor necrosis factor (TNF)-α. Vdelta2(−) βδ T lymphocytes could also kill CMV-infected targets and limit CMV propagation in vitro. Their antiviral reactivity was specific for CMV among herpesviridae and required T-cell receptor engagement, but did not involve class I MHC molecules or NKG2D. Vdelta2(−) γδ T lymphocytes expressed receptors essential for intestinal homing and were strongly activated by intestinal tumor, but not normal, epithelial cell lines. High frequencies of CMV- and tumor-specific Vdelta2(−) γδ T lymphocytes were found among patients' γδ T cells. In conclusion, Vdelta2(−) γδ T cells may play a role in protecting against CMV and tumors, probably through mucosal surveillance of cellular stress, and represent a population that is largely functionally distinct from Vgamma9Vdelta2(+) T cells [46, 47].

The consensus from studies in animal models of experimental infection is that the induction and maintenance of virus-specific CD4+ and CD8+α β + T-cell responses are sufficient and often essential for the resolution of infection [48, 49]. Furthermore, α β+T cells provide immunologic memory that may be of major significance in settings where the patient will experience repeated exposure to the virus or where the virus has established a latent infection in the host [50, 51]. The methodology for isolating polyclonal and clonal populations of α β+T cells with defined specificity for viral antigens is well established for several viruses that cause disease in transplant recipients [52]. Therefore, strategies for developing adoptive cellular immunotherapy for transplant recipients have primarily focused on the use of virus-specific α β+T cells [53, 54].

Experiments performed in animal models to examine the cellular requirements for an effective host response to viral infection have included a detailed analysis of the contributions of class II MHC-restricted CD4+ T helper (TH) cells and class I MHC-restricted CD8+ cytotoxic T lymphocytes (CTLs). Initially, the adoptive transfer of purified T-cell subsets or

T-cell clones to augment individual responses or the administration of monoclonal antibodies to deplete the activity of a single T-cell subset was used as a strategy for the examination of the antiviral activity of CD4+ and CD8+ T cells [53–56]. Later studies used gene knockout mice rendered deficient in expression of class I or class II MHC molecules and that thus were unable to generate CD8+ or CD4+ T cells, respectively [57–59]. The results of these experiments demonstrated that, depending on the dose, route, timing, pathogenesis, virulence, and type of challenge virus, either CD8+ or CD4+ virus-specific T cells could provide protective immunity to the virus challenge and could resolve acute infection [55–59]. This analysis in animal models demonstrated the crucial role of CD8+ and CD4+ antiviral T cells and provided important insights into the cooperation between these subsets. These studies also suggested that the development of adoptive immunotherapy in humans should be guided by an understanding of the antiviral activity of CD4+ and CD8+ T-cell subsets for individual viruses.

48.3 Effector Mechanisms of αβ+ T Cells

Basic studies of T-cell biology have provided insight into the effector mechanisms used by CD4+ and CD8+ T cells to resolve viral infections.

48.3.1 CD4+ αβ+ T Helper Effector Functions

Antigen recognition by virus-specific CD4+ T cells involves an interaction between the αβ+ T-cell receptor and the class II MHC heterodimer containing a peptide fragment derived from a viral protein in its binding groove [60, 61]. The expression of class II MHC is primarily restricted to professional antigen-presenting cells (APCs), such as dendritic cells (DCs), macrophages, and B cells, but it can be induced on other cell types, including endothelial cells. The antigens presented to CD4+ T cells are usually endocytosed by the APCs and then degraded by proteolysis in endosomal compartments where the resulting peptides encounter and bind to class II MHC molecules that traffic to the cell surface. Because the viral antigens can be obtained by endocytosis at the site of infection or in the draining lymph nodes, the APC does not actually need to be infected with the virus to activate the CD4+ TH cells. Several viruses, including human immunodeficiency virus (HIV), CMV, EBV, and measles, do productively infect cells bearing class II MHC molecules, and, in this circumstance, endogenously synthesized viral proteins may also enter the class II processing pathway and then directly activate CD4+TH cells [61, 62].

A primary function of activated CD4+ TH cells is the production of cytokines that orchestrate a local and systemic host response to the pathogen by autocrine and paracrine effects. In addition, CD4+ T cells also condition professional APCs through the interaction of CD40 and the CD40 ligand, which results in APC maturation and superior stimulation of T cells [63, 64]. Mature differentiated CD4+ TH cells can be categorized into TH1, TH2, and a newly discovered TH17 subset with distinct profiles of cytokine production and differing functional roles in the immune response [65, 66]. Interferon-γ (IFN-γ) and interleukin-12 (IL-12) in the milieu favor the development of the TH1 phenotype; IL-4 and IL-10 promote the development of the TH2 phenotype; and IL-6, transforming growth factor (TGF)-β, IL-21, and IL-23 promote the development of the TH17 phenotype [66–69]. The participation of TGF-β in the differentiation of TH17 cells places the TH17 lineage in close relationship with CD4(+) CD25(+)Foxp3(+) regulatory T cells (Tregs), as TGF-α also induces differentiation of naive T cells into Foxp3(+) Tregs in the peripheral immune compartment.

The CD4+ TH1 subset produces IL-2, IFN-γ, and TNF, and it preferentially promotes cell-mediated immune responses [63]. IL-2 activates and induces the proliferation of NK cells, and it is the major growth factor for CD8+CTLs [70–72]. Indeed, in an animal model of persistent viral infection, the ability to mount a virus-specific CD4+ TH cell response was essential for sustaining virus-specific CD8+ CTL responses [73]. IFN-γ and TNF activate nonspecific effector cells, such as macrophages and NK cells, exert direct inhibitory effects on virus replication, and promote the resistance of uninfected cells to viral infection [74, 75]. The TH1 subset of CD4+TH cells produces IL-4, IL-5, and IL-10, and promotes the development of humoral immunity and the activation and differentiation of eosinophils and mast cells [76, 77]. The investigation of the differentiation, effector function, and regulation of TH17 cells has opened up a new framework for understanding T-cell differentiation, and the role of this TH subset in antiviral immunity remains to be elucidated.

Although little information exists concerning the role of distinct CD4+ TH cell subsets in the immune response to individual viral infections in humans, insights derived from murine models suggest that the subtype of CD4+ T cells used in adoptive therapy may affect its efficacy and safety. For example, in a murine model of influenza infection, the transfer of an influenza-specific CD4+ TH1 clone was protective, whereas the transfer of an influenza-specific TH2 clone failed to confer protective immunity [78]. In the RSV model, RSV-specific TH1 and TH2 cells exhibited antiviral activity and promoted clearance of virus, but the TH2 cells induced an eosinophil-rich infiltrate in the lungs and worsened the morbidity [79]. Studies of the antiviral properties of TH17 cells that appear to have a role in autoimmunity and are important for fungal and bacterial infections [80–83] are only now beginning. Recent studies in mice suggest that in some circumstances, a TH17 response may promote viral persistence [84]. These results indicate that, depending on the viral infection being treated, the selection of CD4+ T cells for use in therapy may be a decisive factor in its success or failure.

Tregs defined by the expression of the Foxp3 transcription factor represent yet another T-cell subset that plays a crucial role in balancing inflammation and antigen-specific immune responses. In chronic infectious diseases, Tregs dampen inflammation to limit tissue damage, but they can also inhibit ensuing effector immunity, thereby impairing pathogen clearance. Chronic persistent infections by human pathogens such as parasites, viruses, and (myco) bacteria can all result in the induction of both CD4(+) and CD8(+) Tregs. However, among the many different subsets of Tregs that are induced, mostly CD4(+) Tregs have been studied. A remarkably increased frequency has been observed at the site of infection, supporting a role in pathogen containment. Various populations of DCs are central to the orchestration of this control. Antigen specificity has been demonstrated for several pathogen-derived antigens and the related Tregs. A better understanding of the induction and activity of Tregs is relevant for the design of better vaccines that optimally induce effector immunity without coinduction of excessive Treg activity [85–88].

48.3.2 CD8+ αβ+ Cytotoxic T-Lymphocyte Effector Functions

CD8+ cytotoxic T cells recognize peptides bound to class I MHC molecules on the surface of cells [89]. The antigenic peptides displayed with class I MHC are primarily derived by the proteolytic cleavage of intracellular proteins by the proteosome complex [90] and are then transported from the cytosol into the endoplasmic reticulum (ER) by a heterodimeric protein complex termed the "transporter associated with antigen presentation" (TAP) [91]. In the ER, the peptides bind to the class I MHC heavy chain, leading to the formation of a stable trimolecular peptide–class I H chain–β2 microglobulin complex that is transported via the Golgi apparatus to the cell surface. Because class I MHC molecules are constitutively expressed or they are inducible in most cell types, CD8+ CTLs serve as the surveillance mechanism for detecting and eliminating virus-infected cells; these appear to be required for the clearance and resolution of most viral infections [48].

The interaction between the T-cell receptor and the relevant class I–peptide complex usually results in the direct lysis of the target cell and the production of TH1-type cytokines, including IFN-γ and TNF. However, in contrast to CD4+ TH1 cells, differentiated effector CD8+ CTLs, many of which have lost CD28 expression, produce reduced amounts of IL-2 following antigen stimulation, and they are dependent on IL-2-producing CD4+ TH for proliferation [92, 93]. The lytic signal delivered by CD8+CTLs involves the directed exocytosis of cytolytic granules containing perforin and serine esterases (granzymes) or Fas–Fas ligand (FasL) interactions [94]. Perforin disrupts the cytoplasmic and nuclear membranes of the target cell, facilitating the entry of the granzymes that induce DNA fragmentation and ensuring the destruction of the target cell and the cessation of virus replication. Activated CD8+CTLs also express FasL, and they may induce programmed cell death in virus-infected target cells expressing Fas.

The contribution of cytolytic granules to the antiviral activities of CD8+CTLs has most clearly been demonstrated in the murine lymphocytic choriomeningitis virus (LCMV) model. Mice with a disruption in the perforin gene cannot induce the membrane injury to lyse the target cell [95]. CD8+ T cells from perforin-deficient mice inoculated with LCMV proliferate in response to the virus challenge but exhibit only weak LCMV-specific cytolytic activity in vitro, an effect possibly mediated by Fas–FasL interactions [95]. Perforin-deficient mice fail to clear LCMV infection in vivo, illustrating the necessity for perforin-mediated lytic events for the resolution of this infection [95].

48.3.3 Cytomegalovirus-Specific T-Cell Immunity and Cytomegalovirus Disease in Immunosuppressed Transplant Recipients

CMV is a ubiquitous herpesvirus that infects 50–90 % of the population. Primary infection in immunocompetent hosts is largely unrecognized except as an occasional cause of mononucleosis, and viral persistence is not associated with any clinical sequelae. However, in patients with iatrogenic or acquired immunodeficiency, the reactivation of CMV in CMV-seropositive hosts or the acquisition of primary CMV infection from blood products or the donated organ often leads to progressive infection and visceral disease and represents a major obstacle to a successful outcome for transplant recipients.

The clinical manifestations of CMV infection may differ, depending on the type of transplant and associated clinical factors. SOT recipients who are CMV-seronegative and who receive an organ from a CMV-seropositive donor often develop a CMV syndrome consisting of fever, leukopenia, hepatosplenomegaly, myalgia, and occasionally pneumonitis [96, 97]. Reactivation of CMV in seropositive SOT recipients may also progress to visceral infection, especially in those patients who require intense therapy with immunosuppressive drugs to treat episodes of rejection. Allogeneic HSCT recipients who develop primary infection or reactivation with CMV may also develop fever and leukopenia, but interstitial pneumonitis or enteritis are the most common manifestations of CMV disease in these patients [9].

A critical role for αβ+ T cells in human CMV (HCMV) infection was first suggested by studies in the murine cytomegalovirus (MCMV) model. MCMV is genetically distinct from HCMV, but the pathogenesis of infection in immunosuppressed mice is similar to that for HCMV. In the MCMV model, the transfer of CD8+MCMV-specific CTLs alone was sufficient to protect mice from fatal CMV infection, although the administration of CD4+ TH cells was essential for eliminating salivary gland infection [98–100]. The

administration of CD8+ CTLs specific for the MCMV major immediate–early protein alone were sufficient to provide protective immunity from lethal virus challenge, suggesting that restoring even a limited repertoire of the host CTL response can be therapeutically beneficial [98].

The hypothesis that progressive HCMV infection in HSCT and SOT recipients is related to a quantitative deficiency of virus-specific αβ+ T-cell responses has been examined in several studies. Quinnan et al. [101] showed that the recovery of cytolytic activity for CMV-infected fibroblasts in samples of peripheral blood lymphocytes obtained from allogeneic HSCT and renal transplant recipients was associated with the resolution of CMV infection. Subsequent studies have cultured the peripheral blood lymphocytes from allogeneic HSCT recipients in vitro to distinguish more clearly the recovery of CD4+ and CD8+CMV-specific T-cell responses and to improve the sensitivity for detecting these responses [102, 103]. In all of the published reports, a correlation was observed between the presence of MHC-restricted αβ+ T-cell responses to CMV and protection from the subsequent occurrence of CMV disease, supporting the concept that αβ+ T-cell responses are an essential component of protective immunity to CMV [53, 54].

Analyses using newer technologies developed for the direct quantitation of antigen-specific T cells, such as intracellular cytokine staining or staining with tetrameric human leukocyte antigen (HLA) class I–peptide complexes, have further confirmed the role of CMV-specific T cells in controlling CMV infection. In healthy CMV-seropositive individuals, up to 40 % of all T cells in the peripheral blood can be specific for CMV [104], underlining the importance of a strong CMV-specific cellular immunity for containing persistent CMV infection.

These methods have also been applied to analyses of T-cell responses after allogeneic stem cell transplantation. Fluorochrome-conjugated tetrameric complexes of HLA-A2 molecules loaded with the epitope NLVPMVATV (NLV) derived from the CMV protein pp65 were utilized to quantify CMV-specific CD8αβ+ T cells in HLA-A*0201–positive recipients after allogeneic stem cell transplantation (Figure 48-1). In patients given allografts from a sibling in which both the patient and the donor were seropositive for

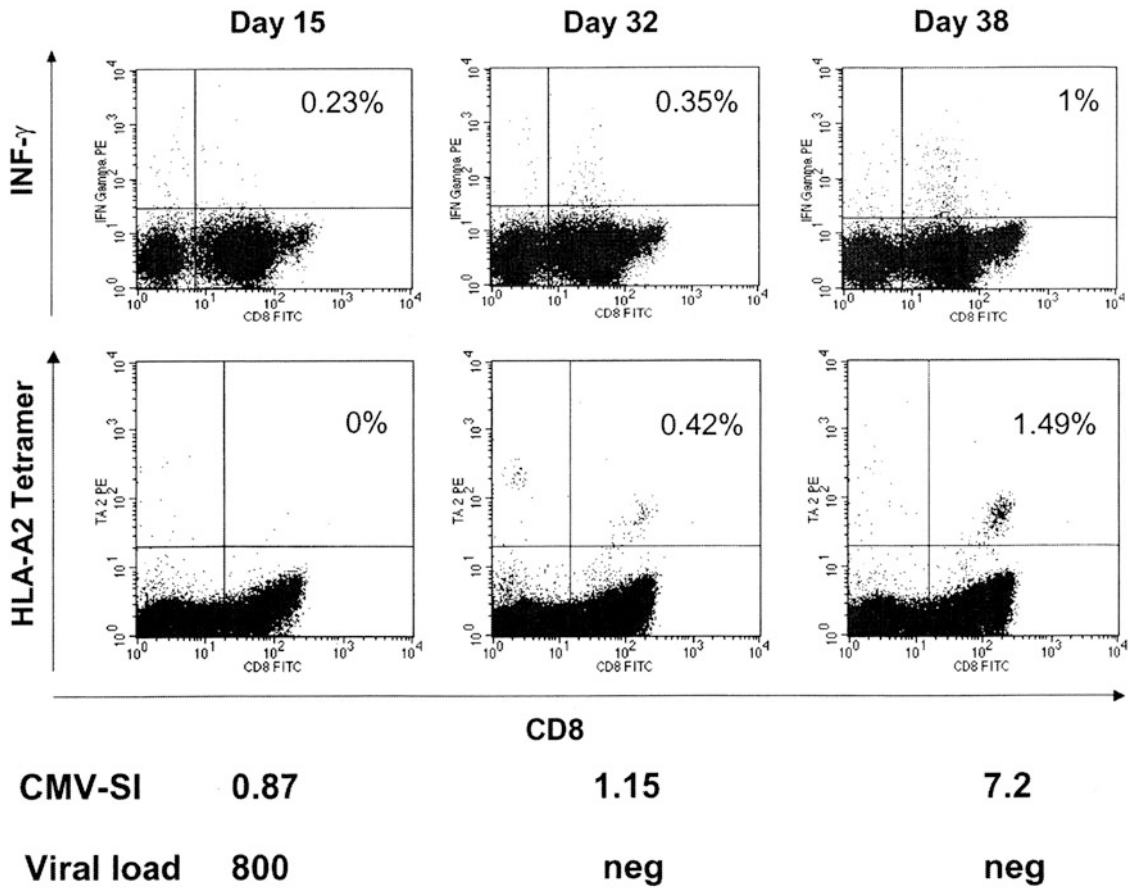

FIGURE 48-1. Reconstitution of cytomegalovirus (CMV)-specific T-cell responses and viral load after T-cell transfer. Patient number 4 had a high viral load at the time of T-cell transfer, but on day 15 after transfer, viral load had decreased to 800 viral copies per milliliter of blood. At day 38 when the virus had been cleared entirely, the reconstitution of CMV-specific lymphoproliferation and CMV peptide-specific A*0201-restricted CD8+ T cells was also shown by intracellular IFN-γ and tetramer staining.

CMV before stem cell transplantation, the recovery of CMV-specific CTLs was rapid, reaching up to 21 % of all CD8αβ+ T cells [105]. Early reconstitution was not observed if either the donor or recipient was seronegative for CMV. In the recipients of transplants from unrelated volunteer donors, the recovery of CMV-specific CTLs was delayed in comparison to that observed in the recipients of transplants from siblings, and no CTLs were observed within the first 100 days after transplantation [105]. Recovery of CMV-specific CTLs to levels greater than 10 cells/μL blood was associated with protection from CMV disease [105, 106]. Furthermore, the absolute number of A2-NLV-specific CD8+ T cells in the grafts correlated inversely with the number of preemptive ganciclovir courses that were administered. In contrast, CMV-seropositive patients receiving a transplant from a CMV-seronegative donor displayed a delayed reconstitution of CMV-specific CD8+ T cells [96]. Thus, the CMV serostatus of the donor, as well as the enumeration of CMV-specific CD8+ T cells in the graft, may identify SCT recipients at risk for developing CMV disease [107].

48.3.4 Specificity of CD8+ αβ+ Cytomegalovirus-Specific T Cells

The CMV genome may encode more than 200 proteins in permissively infected cells, providing a large number of potential antigens that could be presented to CD8+ cytotoxic T cells. Definition of the specificity of CTL responses in individuals with protective immunity to CMV is a critical prelude to the development of effective adoptive immunotherapy to ensure that the T cells selected for use in therapy represent the protective responses in immunocompetent hosts. CMV expresses its genes in a temporal sequence, with discrete phases of gene expression that are called the immediate early (IE), early (E), and late (L) phases [108]. Infected cells expressing a limited array of viral proteins can be prepared by the timed addition of inhibitors that block viral protein or RNA synthesis; this methodology was used to determine whether CTLs preferentially recognized proteins produced at IE, E, or L stages of the replicative cycle. Surprisingly, the introduction of an RNA synthesis inhibitor to target cells just prior to virus exposure to prevent the production of newly synthesized viral proteins after viral entry did not prevent the recognition of these infected cells by CD8+ CMV-specific CTL lines and most CTL clones isolated from healthy CMV-seropositive individuals [109]. This demonstrated that expression of the viral gene in the target cell was not required to sensitize the cell for lysis and that the virion proteins introduced into the target cell cytosol following viral entry, rather than newly synthesized IE, E, or L proteins, were important target antigens of the host CTL response. The specificity of the CD8+ CMV-specific CTLs that recovered after allogeneic bone marrow transplantation and their association with protection from subsequent CMV disease were similarly analyzed, and these CTLs were also specific for epitopes derived from structural virion proteins [108].

The contribution of individual virion proteins as antigens for CTL was assessed by pulsing peptide fragments of purified proteins onto target cells or by infecting target cells with recombinant vaccinia viruses encoding a single CMV gene. Reportedly, the matrix protein pp65 is the most frequent target of the major host response, although strong responses to a second matrix protein pp150 do occur in some individuals [110–112]. Insight into the biologic importance of CTLs for structural virion proteins was suggested by studies evaluating the ability of these CTLs to kill CMV-infected cells at different stages of the replication cycle. CMV-infected target cells are rapidly sensitized (<1 h after virus inoculation) for lysis by CD8+ CTLs specific for pp65 or pp150, and the infected cell remains a target throughout the entire replicative cycle. Thus, these CTLs should be effective in limiting virus dissemination after reactivation by promptly eliminating the newly infected cells. However, the rapid recognition of structural proteins such as pp65 and pp150 is not observed with CTLs specific for virus envelope gB protein. CTLs specific for gB lyse CMV-infected cells poorly at all stages of the replicative cycle, and, in CMV-seropositive individuals, these are present at a substantially lower frequency than are the CTLs for pp65 or pp150 [112, 113]. The fact that CTLs specific for pp65 and/or pp150 are maintained at an extremely high frequency for life in healthy CMV-seropositive individuals suggests that, even in immunocompetent hosts, virus reactivation occurs intermittently but remains subclinical because of rapid control by the host immune response.

An HCMV deletion mutant that is deleted of the four viral genes that are responsible for interfering with class I MHC presentation has also been used for analysis of CMV-specific CTLs in normal CMV seropositive individuals. With this approach, a large fraction of the CD8;+ CTL response was found to be specific for viral antigens expressed during the IE and E phases of virus replication and presented by fibroblasts infected with RV798 but not wild-type CMV [114, 115]. Similar results were obtained with the use of overlapping peptide panels spanning the entire CMV genome [116]. Thus, reconstitution of T-cell immunity in immunodeficient patients by cell therapy or by vaccination may need to target multiple viral antigens to completely restore immunologic control of CMV.

48.3.5 Specificity of CD4+ αβ+ Cytomegalovirus-Specific T-Cell Responses

CD4;+ TH responses to antigen preparations extracted from CMV-infected cells are readily demonstrable in healthy CMV-seropositive individuals. Recombinant gB, pp65, IE2, p52, and IE1 CMV proteins have been generated and used to determine the specificity of CD4;+ TH responses. Detectable

responses occurred to all the antigens studied, with approximately 70% of the individuals tested responding to gB, pp65, and IE2, and a smaller proportion responding to IE1 and the DNA-binding protein p52 [117–120]. This data, and studies with peptide panels [105], suggest that considerable diversity exists in the CD4;+ TH response to CMV.

Animal model studies have illustrated the potential importance of characterizing the cytokine profile of the CD4;+ TH cells that are going to be used in adoptive immunotherapy to improve the safety and efficacy of therapy. The cytokine profile of CD4;+ CMV-specific TH clones isolated from CMV-seropositive individuals has been analyzed; all of the clones tested produced IL-2 and IFN-γ characteristic of TH1 cells, but many also produced IL-4 consistent with an overlapping phenotype. Whether the phenotype of these cells can be fixed to a TH1 or TH2 pattern in vitro, and if this will be necessary for the safety and/or efficacy of immunotherapy with CD4;+ CMV-specific T cells remain to be determined.

48.3.6 Immune Evasion Strategies Used by Herpesviruses

Several viruses have evolved strategies for evading recognition by the host immune response, and the identification of the mechanisms utilized by these viruses should assist in selecting the appropriate effector cells to use for restoration of protective immunity in adoptive immunotherapy. Herpesviruses can enter a latent state that allows the virus to persist even during the height of the immune response, although the cellular sites of latency and the status of viral gene expression in such cells are not completely defined [108, 121].

Once the virus reactivates from latency and initiates replication, the infection is likely to progress in the absence of a host response to limit dissemination. One strategy that CMV uses to elude the destruction of permissively infected cells by CD8+CTLs is to inhibit the cellular processes involved in delivering class I MHC molecules bearing antigenic peptides to the cell surface. Studies of cells replicating CMV have demonstrated a reduced surface expression of class I MHC at the E and L stages of the replicative cycle [101]. The mechanisms involved in the downregulation of class I MHC were elucidated with the identification of four viral genes—US2, US3, US6, and US11—that interfere in a coordinated fashion at discrete steps in the antigen-presentation pathway [122–126]. These virus-encoded proteins collectively impede the efficient presentation of viral proteins expressed after the IE stage of replication and provide a biologic basis for the relative immunodominance of CD8+ CTLs directed against structural virion and IE proteins that are presented before this global blockade in class I antigen presentation.

The IE protein is produced in abundance immediately after infection before the decline in cell surface class I MHC expression. Conceptually, this would be a good antigen to target in adoptive immunotherapy, and IE-specific T cells can be isolated from many CMV-seropositive donors [113]. Surprisingly, IE-specific CTLs fail to lyse permissively infected target cells even if virus replication is arrested at the IE stage of replication [113]. One study demonstrated that the pp65 protein that is introduced into the cytosol after virion entry and before IE synthesis selectively interferes with the presentation of IE peptides [127]. It remains to be determined whether these immune evasion strategies that interfere profoundly with CTL recognition in vitro are as effective in vivo. It would seem prudent to utilize T cells for adoptive therapy that are specific for structural virion proteins and IE proteins since these are typically the dominant responses in healthy donors.

The early and sustained decrease in surface class I MHC expression in CMV-infected cells should enhance their susceptibility to recognition by NK cells and suggests a potential role for this effector population in eliminating permissively infected target cells. Although no homology exists between the genes encoding HCMV and mouse CMV (MCMV) that modulate the expression of class I MHC, both viruses effectively use analogous and redundant gene functions to inhibit antigen presentation [128]; the studies of interactions with NK cells have used murine models. NK lymphocytes monitor the expression of class I MHC molecules by different cell surface receptors, which transduce signals leading to the inhibition or activation of the NK cells. The following three groups of inhibitory NK receptors screen cells for downregulated expression of class I MHC molecules: (1) the killer cell immunoglobulin (Ig)–like receptors in primates, (2) the lectin-like receptors Ly49 in rodents, and (3) the heterodimer of CD94 and NKG2A molecules shared by rodents and primates [129]. However, these three families of class I MHC-specific receptors contain stimulatory receptors as well (e.g., KIR2DS and KIR3DS, Ly49D and Ly49H, and NKG2C and NKG2E). In addition, NK cells express a variety of other receptors that recognize nonclassic MHC molecules and non-MHC-related proteins [130, 131].

Binding of the C-type lectin receptor NKG2 to its ligand can overcome inhibitory signals [132]. The NKG2D homodimer is a potent activating receptor in both humans and mice; it is expressed not only on NK cells but also on activated macrophages (in mice) and γδ+ T cells, where it functions as a coactivating receptor [133]. Murine NKG2D interacts with the following two cell surface ligands that are related to class I MHC molecules: (1) Rae-1 and (2) H-60 [134, 135]. MCMV glycoprotein 40 (gp40) was recently shown to downregulate H60, a high-affinity ligand for NKG2D receptors, thereby inhibiting NK cell activation. The deletion of m152 abolishes this function, rendering otherwise resistant virus-infected cells susceptible to NK cells.

The arsenal of stealth tactics used by HCMV also extends to the evasion of NK recognition. CMV encodes UL18, a molecule that is homologous to class I MHC; when UL18 is expressed on the cell surface, it delivers an inhibitory signal

to the NK cell and prevents NK-mediated lysis [38]. The ligands for NKG2D in humans, such as class I MHC chain-related chain A (MICA) and class I MHC chain-related chain B (MICB), can be induced by cellular stress [136]; malignant transformation; or infection with herpesviruses, including CMV [137]. However, CMV infection downregulates MICB through the expression of UL16 [131, 138], but MICA expression is retained and costimulates NKG2D expressing CMV-specific CD8+ T cells even in situation where class I MHC molecules are downregulated [132].

48.4 Clinical Studies of Adoptive Immunotherapy with Cytomegalovirus-Specific T Cells

The investigation of adoptive immunotherapy with CMV-specific T cells is difficult for some subgroups of transplant recipients. For CMV-seropositive recipients of a stem cell graft from a CMV-seronegative donor generation of CMV-specific T cells is difficult. Isolating and expanding autologous virus-specific T cells before transplantation for use in immunotherapy after SCT is technically difficult because of the extremely low precursor frequency of such cells in the peripheral blood in the absence of prior natural infection. One potential strategy would be to induce virus-specific T-cell responses in the CMV-seronegative recipient or CMV-seronegative donor by vaccination before transplantation and then to isolate these cells for use in therapy as augmentation of the endogenous response during the period of posttransplant immunosuppression. The increasing availability of safe and effective vaccines for eliciting CMV-specific T-cell responses will help to further develop this strategy [138].

There are two alternative approaches: a selection of virus-specific T cells from a partially matched seropositive third-party donor which was successfully demonstrated before but could be hampered but a limited time of persistence following adoptive transfer. CTL lines with partial HLA matches from CTL banks were successfully administered to patients with a PTLD after solid organ transplantation, with a trend of better responses with higher number of CD4+ T cells infused and closer HLA matching between donor and recipient [139]. In addition, successful transfer of CMV-specific T-cell lines from a third-party donor only matched for 1 HLA-allele was reported after haploidentical HCT [140].

Another strategy is to boost endogenous HCMV-specific T-cell reconstitution by donor monocyte-derived DCs pulsed with HCMV peptides as shown successfully in ten patients after allogeneic SCT [141]. No side effects or induction/aggravation of acute GVHD were observed. In the setting of HLA-identical allogeneic HSCT, the CMV-specific T cells that will be used for posttransplantation immunotherapy should be derived from the donor because the immune system that develops in the host after transplantation is derived from donor hematopoietic cells.

Recipients of HSCTs from a CMV-seropositive donor provide the most favorable setting for the investigation of CMV-specific T-cell therapy because T cells with reactivity for CMV antigens can be readily isolated from the donor by in vitro stimulation with autologous APCs expressing CMV antigens. The generation of polyclonal populations of CMV-reactive T cells from the donor is technically feasible, but these polyclonal populations may also contain T cells that are not reactive with CMV, including T cells that could recognize recipient minor histocompatibility antigens. Thus, one concern with the use of polyclonal donor T cells for adoptive transfer is the potential for inducing graft-versus-host disease (GVHD). Indeed, researchers have frequently observed GVHD when polyclonal T cells are administered to HSCT recipients to treat EBV-induced lymphomas [142]. This potential problem could be avoided if individual CMV-specific T-cell clones or highly enriched populations of CMV-specific T cells are isolated and selected.

The first evaluation of adoptive immunotherapy with virus-specific T cells in humans was performed in allogeneic HSCT recipients. The study examined the safety and immunomodulatory properties of administering CD8+ class I MHC-restricted CMV-specific cytotoxic T-cell clones [143]. CD8+ CTLs were selected for initial investigation because the data from animal models of CMV infection and reconstitution studies in humans suggested that CD8+ CTLs were necessary and sufficient for protective immunity [18, 102]. CD8+ CMV-specific T-cell clones were isolated by limiting dilution cloning from polyclonal T-cell lines established from the bone marrow donor. Clones that were αβ+ T-cell receptor, CD3+, CD8+, and CD4+ and that recognized epitopes derived from structural virion proteins in the context of class I MHC were selected for intravenous administration to the recipient. Fourteen patients were treated with four escalating doses of CMV-specific T cells beginning 28–42 days after HSCT. No serious acute toxicities occurred, even at the highest cell dose in any of the 14 patients treated, and the patients did not require hospitalization for the infusions. Two patients experienced minor side effects that included transient fever and chills [144]. CTL responses were evident within 2 days after the first infusion, and they increased with each subsequent infusion such that the responses measured 2 days after the fourth infusion were equivalent to those present in peripheral blood lymphocytes obtained from the healthy donor [144].

Patients who recovered CD4+ CMV-specific TH cell responses maintained strong CD8+ CTL responses. New techniques such as the peptide-MHC multimer technology as well as the cytokine capture assay have been developed that allow the easier selection of CMV-specific T cells for use in therapy. To avoid generation of virus-specific cell lines by repetitive stimulation and long-term in vitro culture which interfers with in vivo persistence and proliferation novel

strategies for direct cell selection without the necessity of long-term culture were developed.

The first technology, which was transferred to the clinic, was the isolation of Ag-specific T cells from the blood of CMV-seropositive donors based on the interferon γ (IFNγ) secretion of T cells after ex vivo stimulation with viral antigens [145]. Using these technologies 18 patients after allo SCT from HLA-mismatched/haploidentical or HLA-matched unrelated donors were treated for chemorefractory CMV-infection with polyclonal CMV-specific T cells generated by ex vivo stimulation with CMVpp65 antigens followed by isolation of IFNγ-producing cells. After the transfer of a mean of 21×10^3/kg CMVpp65-specific T cells 83 % of patients cleared the viral infection or showed a significantly reduced viral load. The viral control was associated with in vivo expansion of CMV-specific T cells in 12 of 16 evaluable patients [146].

Following the introduction of novel multimer techniques such as tetramers and pentamers for the identification and quantitation of antigen-specific T cells, a modification of this technology—the streptamers were established for antigen-specific T-cell selection and transfer under GMP conditions [147, 148]. The high purity of this selection technology allows successful transfer of virus-specific T cells with a very low risk of alloreactivity [148]. In spite of the fact that often only low doses of streptamer-selected donor-derived T cells can be isolated with this technology the strong pathogen-specific T-cell expansion mediated effective anti-HCMV activity [149]. The transfer of CMV-specific CD4+ T cells was shown to be effective in inducing CMV-specific CD8+ T-cell responses and reducing CMV DNA load (Figure 48-2) [145]. This study extended the results of previous studies that suggested that T-cell infusions comprising predominantly the CD4 subset may be efficient for the control of CMV viremia in immunosuppressed patients. A single infusion of T cells resulted in the clearing of CMV viremia in 5 of 7 patients, and a second infusion was associated with resolution of viremia in an additional patient, with 6 of 7 patients achieving resolution of CMV. This study along with others illustrates the critical requirement for the presence of CD4+ T cells in the control of many chronic viral infections.

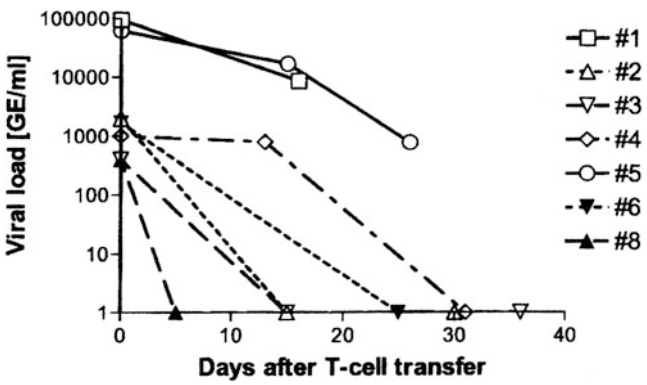

FIGURE 48-2. Decrease in cytomegalovirus (CMV) load following T-cell therapy. The viral load of all seven patients with detectable CMV DNA at the time of T-cell transfer is presented (virus copies per milliliter of blood). In three patients (numbers 1, 5, and 8), virus load increased again; patient number 5 responded to a second T-cell infusion.

48.5 T-Cell Immunotherapy for Posttransplantation Epstein–Barr Virus Infections

EBV infects approximately 90 % of the adult population, and it establishes persistent latent infection in the oropharyngeal and gastric epithelium and in B lymphocytes [150]. The establishment of latency appears to be an important mechanism for immune evasion by EBV. The viral genome persists in latently infected cells as an episome, and three distinct forms of EBV latency have been distinguished on the basis of the expression of different viral proteins [131]. The EBNA-1 protein is expressed in all EBV-associated malignancies, and, therefore, it would be an attractive target antigen for a host T-cell response. However, the expression of EBNA-1 protein is also essential for all three forms of EBV latency, suggesting a mechanism for preventing the presentation of this protein to CD8+ CTLs must exist. The presence of a region containing repetitive sequences of glycine and alanine in the EBNA-1 protein prevents the processing and presentation of EBNA-1, rendering this protein invisible to host CD8+ CTLs [152].

In immunocompetent hosts, EBV infection can be associated with the later development of malignancies, including Burkitt lymphoma, a proportion of cases of Hodgkin disease, and nasopharyngeal carcinoma [137]. These EBV-associated malignancies in immunocompetent hosts typically express very few EBV proteins and exhibit decreased expression of class I MHC molecules and an absence of adhesion molecules to evade immune recognition [153].

In immunodeficient hosts, such as allogeneic SOT and HSCT recipients and patients with acquired immunodeficiency syndrome, EBV infection of B lymphocytes induces their proliferation, and this can progress to a monoclonal immunoblastic lymphoma [154–158]. These EBV-associated lymphoproliferations in immunodeficient hosts typically occur in recipient's B cells in solid organ transplantation and in the B cells from the donor in hematopoietic stem cell transplantation. Several EBV proteins that are targets of the CD8+ CTL response in healthy immunocompetent hosts are expressed in the EBV-infected proliferating B cells, and these cells are not deficient in expression of class I MHC or adhesion molecules. Indeed, the phenotype is strikingly similar to that of EBV-transformed B cells (EBV lymphoblastoid cell lines [EBV-LCL]) that spontaneously grow from blood cultures of EBV-seropositive individuals if T cells are

depleted from the culture or if cyclosporin A is added [159]. Thus, the pathogenesis of EBV lymphomas in immunodeficient hosts appears to reflect the absence of sufficient T-cell immunity to control reactivation of EBV [160], and adoptive immunotherapy with EBV-specific T cells has the potential to restore the requisite effector cells in the host to promote tumor regression [161].

48.5.1 Epstein–Barr Virus-Specific T-Cell Immunity and the Pathogenesis of Epstein–Barr Virus-Induced Lymphoproliferation

The major recognized clinical syndrome of EBV reactivation in immunodeficient hosts is EBV-LPD, which usually occurs in those individuals who are the most severely immunosuppressed. Thus, patients receiving T-cell-depleted bone marrow to prevent GVHD or T-cell-specific antibodies to treat organ rejection or GVHD are at high risk for EBV-LPD [162–168]. Among the recipients of allogeneic T-cell-depleted bone marrow, the risk of EBV-LPD varies from 11 % to 26 %, depending on the method of T-cell depletion and the type of posttransplantation immunosuppression [161, 168]. Recipients of unmodified bone marrow rarely (1 %) develop EBV-LPD unless they require intense posttransplantation immunosuppression with anti-T-cell monoclonal antibodies or antithymocyte globulin (ATG) to treat GVHD.

The type of SOT and the intensity of immunosuppression influence the risk of EBV-LPD in SOT recipients. Those who are EBV-seronegative and who then acquire EBV as a primary infection from the donor organ or blood products are at particularly high risk of developing EBV-LPD after transplantation, presumably because these individuals have no pre-existing T-cell immunity to EBV [158, 169]. The incidence of EBV-LPD increases for all SOT recipients if T-lymphocyte antigen (OKT3) is used as prophylaxis or therapy for rejection episodes [166]. Assessing EBV load by PCR has been found to be useful in predicting the subsequent occurrence of EBV-LPD [170], and interventions using the anti-CD20 antibody or T-cell therapy as immunotherapy are increasingly being used preemptively in patients with a rising EBV DNA load in the peripheral blood [171].

A strong EBV-specific CD8+ cytotoxic T-cell response is elicited in immunocompetent hosts following primary infection with EBV, and it correlates with the resolution of the clinical manifestation of EBV. Reconstitution of EBV-directed T-cell responses is essential for controlling the proliferation of EBV transformed B cells. However, even after autologous peripheral blood stem cell transplantation, EBV-directed cellular immune reconstitution can take up to 6–12 months [172]. Lucas et al. [173] analyzed the temporal recovery of EBV-specific cytotoxic T-cell responses after unmodified or T-cell-depleted hematopoietic stem cell transplantation using EBV-transformed B lymphocytes as stimulator cells [173]. Profound deficiencies of EBV-specific cytotoxic T cells were observed 3 months after transplantation in most individuals, but they were recovered in most patients by 6 months after transplantation [173]. The relative frequencies of T cells specific for different EBV peptides in HSCT recipients closely reflect those of their respective donors.

Most cases of posttransplantation EBV-LPD develop in the first 4 months after transplantation, coincident with the most severe deficiency of EBV-specific CTLs. Moreover, those patients who develop EBV-LPD have weak or undetectable EBV-specific cytolytic activity [167, 168, 173]. These observations combined with the occasional spontaneous regression of established EBV-LPD after a reduction in the intensity of immunosuppressive drug therapy suggest a critical role for T cells in preventing the outgrowth of EBV-infected B cells.

Insights into the roles of different effector populations in controlling EBV-LPD have also been derived from studies in severe combined immunodeficient (SCID) mice inoculated intravenously or subcutaneously with EBV-transformed LCL. Depending on the route of inoculation, these mice develop disseminated or localized EBV lymphomas, and they have been used as models for assessing the roles of αβ+ T-cell subsets and NK cells in promoting tumor regression. In this model, the infusion of CD8+ EBV-specific T-cell lines or clones delayed or completely prevented the outgrowth of EBV lymphoma. CD4+ EBV-specific TH cells also mediated protection in some instances; however, NK or lymphokine-activated killer (LAK) cells were ineffective [174–176]. Studies in mice with established subcutaneous EBV lymphomas have demonstrated that the infusion of EBV-specific CTL lines results in the preferential migration of the infused T cells to the sites of tumors and to tumor regression [177].

48.5.2 Specificity of αβ+ CD8+ and CD4+ Epstein–Barr Virus-Specific Cytotoxic T Lymphocytes

EBV-specific CD8+ CTL responses can be readily elicited from EBV-seropositive donors in vitro using autologous EBV-LCL as stimulator cells. Only a minority of these EBV transformed B cells undergoes full EBV replication and lytic infection. Most cells express only six EBV nuclear antigens (EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, and EBNA-LP) and two membrane antigens (LMP-1 and LMP-2) [153, 154]. These eight EBV antigens are also expressed in the infected B cells in EBV-LPD in immunosuppressed hosts. Vaccinia recombinant viruses encoding these individual EBV proteins have been constructed and used to assess the specificity of EBV-reactive CTLs elicited after the stimulation of peripheral blood lymphocytes with EBV-LCL. In most of the individuals studied, one or more of the EBNA-3A, EBNA-3B,

and EBNA-3C antigens were immunodominant, with lesser responses against EBNA-2, EBNA-LP, LMP-1, and LMP-2 [178, 179]. Notably, EBNA-1 was not recognized as a target antigen by any of the EBV-specific CD8+ CTLs.

Detailed studies of the role of individual EBV proteins expressed in EBV-LCL as target antigens for CD4+ EBV specific TH cells have been performed [151]. In contrast to CD8+ T cells, CD4+ T cells can recognize EBNA-1-positive targets. In healthy EBV-seropositive individuals, 0.5 % of all peripheral blood CD4+ TH1 cells can be specific for EBNA-1, which may represent an interesting target for adoptive immunotherapy with antigen-specific CD4+ T cells [180].

48.5.3 Clinical Studies of Adoptive Immunotherapy with Epstein–Barr Virus-Specific T Cells in Posttransplantation Epstein–Barr Virus Lymphoproliferative Disorder

EBV-LPD occurs more frequently in SOT recipients than in HSCT recipients [167]. However, in allogeneic HSCT recipients, the marrow donor can be used as a source of T cells for immune reconstitution. This strategy was first evaluated in a study by Papadopoulos et al. [142] in which five recipients of T-cell-depleted bone marrow transplants who had developed EBV-LPD received infusions of peripheral blood mononuclear cells (PBMCs) from their EBV-seropositive donors. The dose of T cells administered was 1×10^6 CD3+ cells/kg, and this therapy resulted in the complete regression of EBV-LPD in all five patients within 30 days; three of the five patients became long-term survivors [142]. The authors reported that both acute and chronic GVHD were observed consistent with the presence of alloreactive T cells, as well as EBV-reactive T cells, in the infused cell population. The patients receiving this therapy experienced dramatic increases in circulating EBV-specific CTLs that were consistent with the rapid expansion of this subset after antigen stimulation in vivo. Fourteen additional patients have been treated with cell doses as low as 1×10^5 cells/kg, and 17 of 19 (89 %) of these patients have had complete eradication of the EBV-LPD [176]. The incidence of acute and chronic GVHD was 16 % and 42 %, respectively [176].

The administration of EBV-specific CD4+ and CD8+ T-cell lines that exhibit cytolytic activity for EBV-LCL has been used for the treatment and prevention of EBV-LPD after T-cell-depleted hematopoietic stem cell transplantation [181]. The enrichment of polyclonal CD4+ and CD8+ T cells for EBV reactivity by in vitro culture has been effective in eliminating GVHD as a toxicity of the therapy [182, 183]. In these studies, researchers transduced the T-cell lines with a retroviral vector encoding neomycin phosphotransferase to permit an analysis of the persistence and migration of T cells after adoptive transfer. These studies have demonstrated that the infusion of gene-marked T cells in a dose of 1×10^8 cells/m^2 induces the regression of established EBV-LPD and that it prevents the development of EBV-LPD in susceptible individuals. Moreover, the marked T cells persisted for more than 18 months after infusion, suggesting that the coinfusion of CD4+ and CD8+ T cells may result in the establishment of immunologic memory [182]. In addition, therapeutic efficacy has been demonstrated by the adoptive transfer of EBV specific CTLs to allogeneic unrelated SCT recipients during periods of increasing EBV viral load [31, 184], as well as to patients suffering from chronic EBV infection [185].

For all immunotherapy, a hypothetic concern has been that mutations in virus-specific antigens may lead to viral escape. Researchers reported a patient who developed lymphoma after marrow transplantation in spite of having received donor-derived EBV-specific CTLs. Sequence analysis of the gene coding for the two CTL epitopes in the tumor virus revealed a 245 base pair deletion that had removed these CTL epitopes. Escape mutants may be a serious problem when CTL therapy is directed against unstable tumor cell-derived targets [186].

An updated analysis Doubrovina et al. [187] update the experience of EBV-specific donor-derived adoptive T-cell therapy after allo SCT. Forty-nine patients received EBV-specific T-cell lines from the SC third-party donor on day 4 posttransplant. > 70 % of patients responded associated with a strong proliferation of EBS-specific T cells. The group at Baylor College summarized the experience with the transfer of EBV-specific T cell lines after allo SCT [188]. None of 101 patients who received the transfer prophylactically developed EBV-LPD, whereas 11 of 13 patients treated with EBV-specific T-cell lines for proven LPD achieved a complete remission. Gene marked EBV-specific T cells were detectable up to 9 years after the transfer.

48.5.4 Current Technologies of Enriching Virus-Specific T Cells

48.5.4.1 Selection Strategies for Multipathogen-Specific T Cells

The de novo cell surface expression of the TNF-receptor family member CD137 (4-1BB) or CD154 identifies recently activated, but not resting, human CD4+ and CD8+ memory T cells. Maximum CD137 as well as CD154 expression level is uniformly observed in both T-cell subsets at 24 h after stimulation with antigen [189, 190]. In experiments with CMV- and EBV-reactive T cells the specificity of CD137 and/or CD154 expression by costaining with peptide/HLA tetramers could be confirmed. Substantial proportions of CD137+ as well as 154+ T cells did not produce IFN-γ, suggesting that CD137 and CD154 detects a broader repertoire of antigen-specific T cells. Activated CD137+ and/or CD154T cells could be easily purified by immunomagnetic

selection and expanded in vitro thereafter. This CD137- and/or CD154-based enrichment method was capable of isolating twofold higher numbers of antiviral CD4+ and CD8+ T cells compared to the IFN-γ secretion assay. The CD137 and/or CD154 assay is most attractive for the simultaneous targeting of antiviral TH and effector cells in monitoring studies and adoptive immunotherapy trials.

Using peptide pools covering relevant CMV, EBV, adenovirus as well as Candida- and aspergillus-specific immunodominant antigens we [191] were able to select multipathogen-specific donor T cells using the CD154 selection strategy and depleting efficiently alloreactive T cells.

48.5.5 Obstacles to Effective T-Cell Therapy for Herpesviruses

Despite considerable success in the development and application of T-cell therapy after transplantation, several challenges remain. The efficacy of adoptive immunotherapy in humans can be limited by the failure of Ag-specific T cells, particularly CD8+ T cells, to persist in vivo. Poor persistence may be due in part to the immunosuppressive drugs that many patients receive, but may also reflect intrinsic qualities of the infused T cells, a rather tolerogenic immune environment of the host as well as a lack of support from CD4+ lymphocytes. The pool of lymphocytes from which CD8+ T cells for adoptive immunotherapy can be derived includes naive T cells and antigen-experienced memory T cells, which can be divided into central memory and effector memory subsets that differ in phenotype, homing, and function. Central memory cells express CD62L and CCR7, which promote migration into LNs and proliferate rapidly if reexposed to antigen. Effector memory cells lack CD62L, enabling migration to peripheral tissues, and exhibit immediate effector function. In response to antigen stimulation, both memory subsets proliferate and differentiate into CD62L-cytolytic effector T cells that express high levels of granzymes and perforin but are short lived. Thus acquisition of an effector phenotype during culture has been suggested as a reason for the poor survival of transferred T cells.

Recent work in nonhuman primates has demonstrated that the derivation of effector T-cell clones affects their persistence after adoptive transfer, and the ability to establish durable immune memory. CMV-specific CD8+ T-cell clones derived from central memory T cells, but not effector memory T cells, persisted long-term in vivo, reacquired the phenotype and function of memory T cells, and occupied memory T-cell niches. These results demonstrate that clonally derived CD8+ T cells isolated from central memory T cells are distinct from those derived from effector memory T cells and retain an intrinsic capacity that enables them to survive after adoptive transfer. These results could have significant implications for the selection of T cells to expand or to engineer for adoptive immunotherapy of human infections or malignancy [192]. New strategies using sequential positive and negative selection based on the streptamer technology allow to successfully enrich for antigen-specific central memory T cells which will allow long-term engraftment after adoptive transfer to the patient.

An additional issue is that isolating donor-derived T cells for immunotherapy remains difficult when the allogeneic donor lacks specific immunity, such as in the case of seronegative recipients of EBV-seropositive organ grafts or of CMV-seropositive patients receiving a stem cell graft from a CMV-seronegative donor.

48.5.6 Potential Future Application of Adoptive T-Cell Therapy for Other Infectious Complications After Transplantation

48.5.6.1 Adenovirus Infection

Adenoviruses can establish acute and persistent infections that are generally mild and subclinical in the healthy population but that can be severe or even fatal in immunocompromised hosts. The importance of adenovirus as a cause of disseminated disease has remained underappreciated. Recently, however, the overall importance of this virus has been emphasized, primarily in pediatric patients after stem cell transplantation, and studies of host immunity to adenovirus have been initiated.

An intracellular cytokine secretion assay revealed adenovirus-specific T cells in 171 healthy control persons and 59 immunosuppressed long-term renal transplant recipients [194]. The responding T cells were CD4+ TH cells that showed a considerably homogenous expression of markers characteristic of antigen-experienced memory and/or effector cells of the TH1 phenotype. Using the cytokine-capture assay, adenovirus-specific CD4+ and CD8+ T cells were successfully isolated from stem cell donors [193] and transferred to patients with refractory adenovirus infections posttransplant [194]. Increasing knowledge about the T-cell epitope for adenovirus-specific T cells will further help to improve monitoring and manipulation of adenovirus-specific immune responses posttransplant [195–198].

In a recent publication [199] for 30 patients with Adv disease and/or viremia Adv Hexon-specific T cell lines were selected by stimulation with Hexon-derived peptides and the cytokine catch assay. Complete viral clearance was observed in 86 % of patients with Adv-specific T cell responses. Again after cell selection based on the cytokine-catch assay no acute toxicity or induction of GvHD was observed.

48.5.6.2 Fungal Infections

T cells play a potential role in the host defense against fungal infections. Recently, CD34-derived DCs were shown to

demonstrate an increase in the fluorescent intensity of HLADR, CD80, CD86, and CD54, as well as an increased production of IL-12 upon exposure to *Aspergillus fumigatus* [200]. The activated DCs stimulated proliferation of the autologous lymphocytes, producing high levels of IFN-γ but not IL-10. Moreover, DCs generated from CD34+ progenitors collected prior to stem cell transplantation also partially restored the in vitro antifungal proliferative response of lymphocytes obtained from patients at 1 month after transplantation, indicating that ex vivo-generated DCs may be useful in restoring or enhancing the antifungal immunity after stem cell transplantation.

In the mouse model, the group from Luigina Romani has demonstrated that pulmonary DCs are able to internalize the conidia and hyphae of *A. fumigatus* through distinct phagocytic mechanisms and recognition receptors, discriminate between the different forms of *A. fumigatus* in terms of cytokine production, undergo functional maturation upon migration to the draining lymph nodes and spleen, and instruct local and peripheral TH cell reactivity to the fungus [201]. The phagocytosis of the conidia induced IL-12 production, whereas that of hyphae induced IL-4 and IL-10 production. The same group reported that DCs transfected with yeast or hyphal RNA induced protective immunity to *Candida albicans* in a hematopoietic transplantation model. DCs transfected with yeast but not hyphal RNA expressed fungal mannoproteins on the surface, underwent functional maturation, and produced IL-12 but not IL-4. These cells were also capable of inducing TH1-dependent antifungal resistance when they were delivered subcutaneously in nontransplanted mice in vivo, and they helped to accelerate the functional recovery of *Candida specific* IFN-γ-producing CD4+ donor lymphocytes in mice that underwent allogeneic bone marrow transplantation. These results indicate the potential efficacy of DCs pulsed with fungal RNA as a fungal vaccine [202].

Researchers have investigated the importance of cytokine dysregulation as a risk factor for the development of invasive fungal infections in a murine haploidentical bone marrow transplantation model [203]. At 2 weeks after transplantation, high levels of TH2 cytokines and impaired production of TH1 cytokines were associated with a high susceptibility to disseminated and mucosal *C. albicans* infections, whereas at 5 weeks after transplantation a predominant production of TH1 cytokines was associated with resistance to infection. In addition, the therapeutic ablation of IL-4 and IL-10 increased resistance to invasive candidiasis. The potential additive antifungal effect of adjuvant treatment with immunostimulatory cytokines or the antagonism of TH2 cytokines is further supported by clinical observations, such as the successful management of rhinocerebral zygomycosis in non neutropenic patients by a combination of granulocyte-macrophage colony-stimulating factor, antifungal therapy, and surgical treatment [204].

With the growing body of evidence that T cells are important in the host defense against *Aspergillus* T cells, techniques to select and adoptively transfer anti-*Aspergillus* TH1 cells are being evaluated to reduce infectious mortality in HSCT recipients. Rapid methods for the clinical-scale generation of functionally active anti-*Aspergillus* T cells according to GMP conditions using *Aspergillus* antigens are being developed [205]. *Aspergillus*-specific T cells can also be selected using the IFN-γ secretion assay [205] as well as multipathogen-specific T cells including Candida- and Aspergillus-specific T cells [191, 206]. Recent experiments [207, 208] indicate that also NK cell infusions might mediate antifungal activity and would be applied clinically.

The Perugia group successfully transferred *Aspergillus-specific* T cells to patients after haploidentical HSCT with documented invasive aspergillosis and demonstrated improved control of this devastating infection when compared to historical control patients receiving antifungal pharmacological treatment [209].

48.6 Summary

Considerable progress has been made in understanding the immunobiology of viral infections in immunocompromised hosts. Insights derived from animal models and human studies have provided the rationale for investigating immunotherapy with αβ+ T cells to restore responses considered essential for protective immunity to CMV and EBV. Ongoing studies will address the role of adoptive immunotherapy in the prevention and treatment of adenovirus and invasive fungal infection. The use of genetically modified T cells has been evaluated clinically, and it offers the potential for improving the safety and efficacy of immunotherapy and for removing obstacles to its successful use. Although these studies are in the early stages and they present considerable technical challenges, the results suggest that cellular immunotherapy will be a fruitful area for investigation in the future.

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Part X
Hot Topics

49 Emerging and Rare Viral Infections in Transplantation

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Viral infections are common following solid organ and hematopoietic stem cell transplantation, as detailed in other chapters. While cytomegalovirus (CMV) remains the most prominent virus in transplantation, and the clinical manifestations and complications of infection with other herpesviruses (e.g., herpes simplex virus, Epstein–Barr virus, and human herpesviruses 6 and 8) are well described, improvements in diagnostic techniques have led to the recognition of a number of additional viruses with potential pathogenicity in the immunocompromised host. Outbreaks of emerging viruses, the resurgence of vaccine-preventable viral infections, and the identification of viruses which cause self-limited infection in immunocompetent children but significant disease in transplant recipients have highlighted the breadth of pathogens in this patient population. Some of these emerging and unusual viral pathogens are discussed in alphabetical order below.

49.1 Astrovirus

Astrovirus is a common cause of viral gastroenteritis throughout the world and has been a cause of outbreaks of diarrheal disease in schools, hospitals, nursing homes, and military bases [1–3]. Several recent reports have highlighted the impact of this RNA virus on immunocompromised hosts. In addition to its role in gastroenteritis in these patients, one astrovirus subgroup (VA1/HMO-C) has been reported to cause encephalitis in allogeneic hematopoietic stem cell transplant (HSCT) recipients and children with X-linked agammaglobulinemia [4, 5]. Molecular techniques including reverse-transcription polymerase chain reaction (RT-PCR), RNA sequencing, and next-generation sequencing have demonstrated the presence of this subgroup in the cerebrospinal fluid (CSF) and brain tissue of infected patients. Immunohistochemical staining on biopsy tissue has confirmed the presence of invasive infection. There are no known antiviral treatments available, and central nervous system (CNS) infection has been fatal in the cases reported to date. Additional study is needed to determine the prevalence of astrovirus infection in transplanted patients.

49.2 Bocavirus

Bocavirus is a human parvovirus that causes upper and lower respiratory tract infection, gastroenteritis, and encephalitis in children [6, 7]. Infection is most common in the late fall and winter, and most commonly presents with rhinorrhea, fever, cough, wheezing, or diarrhea. Thirty percent of children develop hypoxia, and a variety of radiographic findings have been reported, including peribronchial cuffing, lobar infiltrates, and pleural effusions. Nosocomial infection has occurred [8]. Bocavirus infection has been reported in the first few weeks following hematopoietic stem cell transplantation, presenting with fever, rhinorrhea, cough, diarrhea, and hypoxia [9]. Virus has been detected in high quantities in plasma, nasopharyngeal aspirates, and stool. Fecal shedding occurs for several weeks to months after clinical resolution of infection [10]. Severe and prolonged diarrhea has been described in liver transplant and hematopoietic stem cell recipients [11]. It has been suggested that bocavirus, like respiratory syncytial virus (RSV) and parainfluenza, may play a role in the development of bronchiolitis obliterans, a manifestation of chronic rejection in lung transplantation [12–14]. To date, there are no data on antiviral efficacy against bocavirus.

49.3 Chikungunya Virus

Chikungunya virus, a mosquito-borne alphavirus transmitted by *Aedes aegypti* and *Aedes albopictus*, is a tropical infection which has caused epidemic disease in India, Thailand, Malaysia, Madagascar, and Reunion Island [15, 16]. It is endemic in eastern, central, and southern Africa. In 2013, chikungunya was reported in St. Martin, with epidemic

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spread throughout the Caribbean, Central America, South America, and Florida, where infection spread locally via *A. aegypti* [17].

After an incubation period of 2–4 days, infection presents with high fever, headache, myalgias, and arthralgias, and can resemble dengue. Arthralgias are typically symmetric and involve large joints, particularly in the legs and arms. Frank arthritis may also occur in the interphalangeal joints, wrists, and ankles. Half of patients also develop a rash, which can be maculopapular, petechial, or bullous and is most commonly located on the trunk, with occasional involvement of the face, extremities, palms, and soles. Ocular pain has also been reported. Rarely, meningoencephalitis, myocarditis, or hepatitis can occur. Symptoms resolve in 7–10 days, although arthralgias and joint stiffness may persist for weeks to months after fever resolves. Severe manifestations of infection with fatal outcomes have been reported in patients with underlying diabetes, lung disease, or chronic neurologic conditions.

Laboratory findings include lymphopenia, thrombocytopenia, elevated transaminases, and hypocalcemia. Diagnosis may be made serologically or by RT-PCR. IgM antibodies develop as fever resolves, typically 1 week after symptom onset. There is currently no known effective antiviral therapy for chikungunya.

During a widespread outbreak of infection on Reunion Island in the Indian Ocean, organ and tissue donors were screened for the presence of chikungunya infection [18]. Corneal donors were found to have serologic and PCR evidence of infection in serum and corneal tissue. Transmission of infection with corneal transplantation is presumed to occur. There have been no reports of transmission of chikungunya in solid organ or stem cell transplantation to date although with reports of infection in Asia, Europe, and North America in travelers from endemic areas, the risk of transmission and the clinical course of infection in these patients require further study.

49.4 Coronavirus

In February 2003 a worldwide outbreak of severe respiratory infection occurred, infecting more than 8000 patients over several months in 29 countries, most severely affecting southern China, Hong Kong, and Canada, with well-described healthcare-associated outbreaks [19–26]. Eighty percent of those affected were previously healthy, with no comorbid conditions. The outbreak began in Guangdong Province, China, in November 2002 and with global travel spread rapidly to multiple continents. The infection of numerous health care workers and the rapidly fatal course of infection, even in healthy hosts, were remarkable. Named Severe Acute Respiratory Syndrome (SARS), this infection was quickly determined to be due to a new strain of coronavirus, a group of viruses known to cause human disease since the 1960s [27].

Patients initially noted high fever, myalgias, headache, and cough, and subsequently became dyspneic [19, 20, 25, 28]. A productive cough was seen in nearly one third of patients, while rash and lymphadenopathy were absent. Lymphopenia, thrombocytopenia, mild elevation of transaminases, prolonged prothrombin time with elevated D-dimers, elevated lactate dehydrogenase (LDH) and creatine kinase (CK), and hyponatremia were common lab findings [25]. Chest radiographs revealed focal airspace consolidation or ground glass opacities, initially without the interstitial infiltrates most characteristic of viral pneumonitis, with lower lung field predominance [22, 25]. Pleural effusions and mediastinal lymphadenopathy were generally absent. Histopathologic findings in lung biopsies and at autopsy included diffuse alveolar damage consistent with adult respiratory distress syndrome (ARDS), with significant alveolar edema, minimal inflammation, and no viral inclusions.

Treatment included corticosteroids and intravenous or oral ribavirin. Although published data are not yet available in humans, animal models suggest that monoclonal antibody to SARS coronavirus (SARS-CoV) is effective in decreasing viral replication and improving outcomes [26]. The overall case fatality rate during the SARS epidemic was nearly 10% [19]. A novel coronavirus was rapidly isolated and identified as the cause of SARS and sequenced, allowing for RT-PCR and serologic testing to be developed [29, 30].

During the SARS outbreak in Toronto, a liver transplant recipient was fatally infected while visiting a medical center for an outpatient clinic visit nearly 10 years posttransplant [31]. Disseminated infection was described in a lung transplant recipient in whom virus was detected in lungs, bowel, lymph nodes, liver, kidney, skeletal muscle, and brain at autopsy [32, 33]. Tissue viral loads were significantly higher in transplant recipients than in their immunocompetent counterparts [34]. The last of the nearly 8000 reported cases of SARS-CoV was reported in May 2004, after which no additional cases have been reported, for unclear reasons.

In September 2012, initial reports of infection with another novel human coronavirus began in Saudi Arabia, with rapid spread to neighboring Egypt, Iran, Jordan, Kuwait, Lebanon, Qatar, Oman, Yemen, and the United Arab Emirates, then to other continents with airline travel [35]. Middle East Respiratory Syndrome Coronavirus (MERS CoV) has been reported to cause severe respiratory tract infection in adult patients, with a mortality rate as high as 60%, most commonly in those with diabetes mellitus and end stage renal disease [36]. After a median incubation period of 5 days (range, 2–14 days), patients often present with fever, cough, dyspnea, and diarrhea after close contact with an infected case and/or travel from an area where infection is active. Coryza, headache, nausea, vomiting, and abdominal pain have also been reported [37]. Laboratory findings include thrombocytopenia, leukopenia, lymphopenia, and elevated

transaminases and LDH. Coinfection with other respiratory viruses has been reported [38]. As with SARS-CoV, health care workers are at risk for infection [39, 40]. Dromedary camels have been reported to harbor infection in the Arabian Peninsula, although the mode of transmission of infection has not yet been elucidated [41].

Several cases of MERS CoV infection have been reported in hematopoietic stem cell and solid organ transplant recipients, who have developed bilateral pulmonary infiltrates with respiratory failure, acute renal failure, leukopenia, thrombocytopenia, and elevated transaminases, at times without fever [36, 42].

While difficult to grow in cell culture, MERS CoV may be diagnosed by RT-PCR on respiratory secretions. Virus has been detected with these techniques in urine and stool as well. To increase the yield of testing, it is recommended that multiple specimens from different sites (e.g., nasopharyngeal swab, sputum, BAL fluid, serum, and stool) be tested using RT-PCR, which is available from the CDC and local health departments in the USA [37]. Due to the risk of transmission of infection to health care workers, contact and airborne precautions are recommended in caring for the suspected MERS-CoV infected patient [39, 40].

While there have been no randomized, controlled clinical trials of antivirals against MERS-CoV, ribavirin and mycophenolate mofetil (an immunosuppressive agent used commonly in transplantation) have in vitro activity against the virus [43]. Ribavirin (in combination with interferon α-2b) has demonstrated promise in decreasing lung injury and viral replication in rhesus macaques infected with MERS-CoV [44]. A retrospective cohort study describing the use of ribavirin and interferon α-2a in twenty patients with severe infection demonstrated an early survival benefit [45].

Whereas coronaviruses made world headlines with the SARS epidemic in 2002–2004 and the MERS-CoV emergence in 2012, coronaviruses OC43 (group 1) and 229E (group 2) have been known for decades to cause upper respiratory tract infections during the fall and winter months. Coronavirus NL63 (group 1) has been reported to cause upper and lower respiratory tract infections in immunocompetent hosts in the Netherlands, and coronavirus HKU1 (group 2) has been reported to cause pneumonia in Hong Kong and France [21]. Non-SARS coronaviruses have recently been associated with severe lower respiratory tract infections in hospitalized patients, including lung and liver transplant recipients [46]. Coronavirus 229E has been isolated from hematopoietic stem cell transplantation recipients with fever and cough associated with interstitial and alveolar pulmonary infiltrates [46]. Pancytopenia may be present. Radiographic infiltrates are most commonly interstitial, although 28% are alveolar. Pleural effusions may be present, and pneumothorax has been noted in a minority of patients. Diagnosis may be made by culture in human hepatoma HUH7 cell line, or by RT-PCR [46, 47].

49.5 Hepatitis E

Hepatitis E is endemic in developing countries and has been reported to cause epidemic disease in Asia, Africa, and Latin America via fecal–oral transmission [48]. Travel-related infection has been reported in those returning from endemic areas with poor sanitation. Recent reports have highlighted the important role of this infection in transplant recipients.

Hepatitis E virus (HEV) is an RNA virus with four major genotypes with presumed reservoirs in pigs, wild boars, deer, and mollusks [49, 50]. Seroprevalence surveys indicate that infection in blood donors, even in France and the USA, is significant; in some areas, hepatitis E is more prevalent than hepatitis A [51, 52]. Epidemics of infection have been described from ingestion of contaminated water, mollusks, and undercooked deer, boar, or pig meat [53–55]. Blood transfusion-transmitted infection has also been described [56–59]. After an incubation period of two to nine weeks, patients develop jaundice, abdominal pain, anorexia, and nausea. Fever and chills may occur as well, although rash is unusual. Diagnosis can be made by RT-PCR detection of HEV RNA, which is present between 2 and 6 weeks after infection, as symptoms occur [60]. IgM antibodies develop as symptoms resolve, approximately 4 weeks after infection. Elevated transaminases occur, peaking approximately 6 weeks after infection. While viremia resolves within 6 weeks of infection, virus remains detectable in stool for several weeks after viremia resolves and IgG appears. Serum IgG antibodies persist for years after acute infection.

Approximately 10% of patients with acute HEV infection develop fulminant hepatitis with acute hepatic failure; the presence of pregnancy or underlying chronic liver disease (e.g., chronic hepatitis C infection or cirrhosis) increases the risk for severe infection [61, 62]. Histopathologic findings on liver biopsy include lymphocytic infiltration of portal triads. Chronic hepatitis appears to be rare in immunocompetent hosts.

Disease in organ transplant recipients has been characterized by a high incidence of chronic infection (in up to 60% of acutely infected patients) with progressive fibrosis and eventual cirrhosis [63–66]. Reactivation of infection has been described in liver and allogeneic HSCT recipients, in whom nearly half of infections became chronic [67–69]. Liver transplant recipients appear to be at increased risk for chronic infection resulting from reactivation of HEV after transplantation, as well as acute graft hepatitis from reactivation or primary infection [70]. Extrahepatic manifestations of infection in transplant recipients have included glomerulonephritis and neurologic involvement [69, 71].

There are no FDA-approved therapies for HEV infection, although decreasing immunosuppression appears to have helped control viremia in some chronically infected transplant recipients. In small studies, interferon alpha and ribavirin have been reported to decrease viremia in these patient populations [72, 73].

49.6 Lymphocytic Choriomeningitis Virus

Lymphocytic choriomeningitis virus (LCMV) gained notoriety as a pathogen in solid organ transplantation in 2005, when the first two outbreaks of donor-transmitted infection were described [74–76]. Additional donor-transmitted outbreaks have recently occurred in the USA and Australia [77–80]. Four clusters of donor-derived infection have occurred in the USA to date.

LCMV is a rodent-borne Old World arenavirus that causes asymptomatic or mild, self-limited illness in the immunocompetent host. Rodents, especially common house mice, laboratory mice, and hamsters, often acquire infection congenitally, resulting in lifelong, asymptomatic excretion of virus in urine, saliva, and feces [81–84]. Human infection occurs via direct contact with infected rodents or aerosolized infected excreta (e.g., with cleaning soiled cage bedding). Symptoms described in immunocompetent humans include fever, headache, and myalgias, with CSF findings consistent with aseptic meningitis (e.g., lymphocytic pleocytosis). In the normal host, infection is self-limited and carries a mortality rate of less than 1% [85].

In the transplant clusters, infection with LCMV has been fatal in more than 80% of cases [74, 78, 80]. Patients have presented within the first month posttransplant with fever, diarrhea, abdominal pain, and dyspnea. Rash, headache, lethargy, hypotension, and the presence of pulmonary infiltrates are variable. Thrombocytopenia and anemia have been present, with variable peripheral leukocyte and lymphocyte counts. Acute hepatitis with elevated transaminases has been noted, as well as coagulopathy with prolonged protimes. Patients have developed rapidly progressive multisystem failure with encephalopathy prior to death. In one cluster, while the donor had no evidence of infection in multiple tissues tested, a pet hamster present in the donor's home for several weeks prior to donation was found to have LCMV in multiple tissues [74]. Virus isolated from the hamster was identical to that isolated from the infected transplant recipients. The survivor in that cluster, a kidney recipient, was treated with discontinuation of all immune suppression except corticosteroids and with intravenous ribavirin. Similar approaches have been used in more recent cases [80].

With four donor-derived infection outbreaks in the USA alone, LCMV infection is likely more common than previously recognized in transplant recipients. Detailed workup of potential organ donors with aseptic meningitis or meningoencephalitis may prevent transmission in some cases. Whether LCMV infection occurs posttransplant in recipients with exposure to pet hamsters or house mice is unknown.

49.7 Metapneumovirus

Human metapneumovirus is a single-stranded RNA paramyxovirus of worldwide endemicity that causes respiratory tract infection in children, the elderly, and immunocompromised adults, with outbreaks reported in long-term care facilities [86–90]. Infection occurs in the late winter and early spring (January through April), similar to the seasonality of respiratory syncytial virus (RSV). Upper and lower respiratory tract symptoms, including rhinorrhea, sore throat, cough, dyspnea, and fever, have been described.

Infection has been described following lung and heart–lung transplantation, resulting in acute pneumonia with diffuse alveolar damage and hyaline membrane formation [91, 92]. Lung transplant recipients with metapneumovirus pneumonia have a 14% mortality rate and are at higher risk for acute and chronic rejection [92, 93]. In renal transplant recipients, pneumonitis due to metapneumovirus has been reported 3 years posttransplant [94]. In one study of HSCT recipients, human metapneumovirus was isolated via RT-PCR in 26% of symptomatic patients undergoing bronchoscopy and carried a mortality rate of 80% [95]. Infection occurred within the first few weeks following transplant, and was characterized by fever, nasal congestion, and cough, with rapid development of hypoxia, hypotension, and progressive pneumonia, with diffuse alveolar hemorrhage in three of five patients [94–96]. Pleural effusions and nodular infiltrates may be seen, which may help differentiate infection from RSV. Coinfection with RSV, rhinovirus, and CMV has been described following lung transplantation [97].

Ribavirin has been demonstrated to decrease human metapneumovirus replication in the lungs in a mouse model [98], and intravenous ribavirin has been effective in the treatment of several lung transplant recipients with metapneumovirus infection [99].

49.8 Measles

Measles outbreaks have occurred in multiple states in recent years, with an attack rate of greater than 90% among susceptible patients, including unvaccinated children and adults [100–108]. Affected patients develop fever, cough, and coryza, associated with a characteristic rash. Infection may be complicated by pneumonia, encephalitis, or dissemination, with significant mortality noted in solid organ and HSCT recipients [109]. Infection has been associated with waning immunity and is diagnosed serologically. There are no data on antivirals for treatment of measles.

49.9 Mumps

Mumps has been increasingly reported in the USA, with more than 10,000 cases reported in a large multistate outbreak in 2005–2006 [110–116]. Patients present with acute onset of unilateral or bilateral parotitis; infection may be complicated by orchitis, oophoritis, pancreatitis, mastitis, meningitis, and encephalitis [117]. Infection may be diagnosed serologically or via PCR [118, 119]. No antivirals have been investigated in the treatment of mumps. Enhanced efforts at immunization against measles and mumps pretransplant as well as active surveillance posttransplant are warranted.

As a result of the re-emergence of these vaccine-preventable viruses, recent guidelines suggest vaccination with the measles, mumps, and rubella (MMR) vaccine 2 years following hematopoietic stem cell transplantation in patients without evidence of graft-versus-host disease [120, 121]. If at all possible, patients undergoing solid organ transplantation who do not have evidence of protection against measles and mumps (e.g., positive IgG antibody to each) should be vaccinated prior to the initiation of immunosuppressive therapy.

49.10 Norovirus

Noroviruses are caliciviruses that cause over 20 million cases of gastroenteritis annually in the USA and over half of all epidemics of gastroenteritis worldwide [122–129]. Infection is acquired via consumption of contaminated foods (including raw oysters, fruit, and vegetables) or via ingestion of or swimming in contaminated water, with spread via fomites and from person to person [130–145]. Infection is extremely contagious and often spreads rapidly as a result of prolonged fecal shedding in affected patients after resolution of symptoms. Outbreaks of infection have been described in multiple settings including military barracks, restaurants, hospitals, long-term care facilities, schools, and cruise ships [122].

Infection may be asymptomatic or present with the sudden onset of nausea, vomiting, and diarrhea after an incubation period of less than 48 h. Some studies have suggested that vomiting is more common in children, with diarrheal symptoms predominating in infants and adults [138, 148]. Infection is most common in the winter months, with symptoms lasting 1–7 days [122, 142, 148]. Attack rates in some outbreaks have been 50–90%, with health care workers at substantial risk for infection [127, 130, 137–139, 144–147].

Noroviruses cannot be cultured in vitro, but RT-PCR and enzyme immunoassay (EIA) assays are available for diagnosis in stool specimens [148–150].

Norovirus infection in solid organ transplant recipients is common, and marked by risk for chronic and relapsing infection [150]. Infection presents with watery diarrhea, which can cause volume depletion and acute renal failure in renal transplant recipients [126, 142, 149, 151]. Patients may be symptomatic for months and may shed virus in stool for years. Hematopoietic stem cell transplant recipients have been reported to develop acute and chronic diarrheal disease from norovirus infection, which has been associated with the subsequent development of chronic GVHD [151, 152]. Receipt of cord blood, induction with fludarabine, and receipt of alemtuzumab have been reported to be risk factors for norovirus infection in this setting. Nosocomial outbreaks of infection in HSCT units have resulted in infection of staff and patients, with sepsis from bacterial translocation complicating several cases [152, 153].

Treatment of norovirus infection in transplant recipients has not been investigated in randomized, controlled trials to date. Reduction of immunosuppression resulted in clearance of infection in one intestinal transplant recipient with norovirus infection [154]. There are no available antiviral therapies to date. Noroviruses are highly resistant to disinfectants, propagating prolonged transmission in many environments.

49.11 Parvovirus B19

Parvovirus B19 infection is common, with 60–90% of adults having serologic evidence of prior infection [155]. In children, parvovirus infection causes erythema infectiosum, a febrile illness with a characteristic “slapped cheek” rash. Adults with acute parvovirus infection develop a flu-like illness, sometimes with resultant arthropathy. A pathogen of erythroid progenitor cells, parvovirus B19 causes severe anemia in patients with underlying hemolytic disorders and hydrops fetalis in pregnancy. In recent years, neurologic involvement including meningoencephalitis has been described, which may be more common in immunocompromised hosts [156, 157].

In transplant recipients, anemia is the most common presentation of infection. Fever occurs in 25% of patients and arthralgia or rash occurs in less than 10% of those affected [158]. Pancytopenia may be present. Other manifestations described in the transplant population include hepatitis, myocarditis, pneumonitis, encephalitis, meningitis, peripheral neuritis, and collapsing glomerulopathy [155, 157–160]. Those with CNS infection may develop sequelae including seizures, cognitive deficits, stroke, and muscle wasting [157]. Donor-transmitted infection has been described, presenting with allograft dysfunction, fever, arthralgia, and pancytopenia, often without a rash [161–164]. Chronic or recurrent anemia may be seen posttransplant, as well as pure red cell aplasia [165, 166]. Parvovirus B19 infection has also been associated with the subsequent development of thrombotic microangiopathy in kidney transplant recipients, including a cluster of cases in Iran; hemophagocytic lymphohistiocytosis has also been described in this population [167, 168]. The significance of the frequent finding of parvovirus DNA in renal allografts pre- and posttransplant is under investigation [169]. In other transplant populations, parvovirus may be associated with chronic cellular allograft rejection [170].

Diagnosis of parvovirus B19 infection may be made by serology, PCR, or bone marrow examination in immunocompetent hosts. The yield of serologic testing (especially IgM) is limited in transplant recipients who may not mount an adequate antibody response to infection, so that RT-PCR on blood, bone marrow, or other involved tissues is necessary to detect infection in many cases [155].

Infection may respond to intravenous immunoglobulin (IVIg), with relapses occurring in up to 25% of immunosuppressed hosts [155]. There are no published data on the use of antivirals in parvovirus infection.

49.12 Polyoma Viruses (KI, WU, and Merkel Cell Carcinoma Polyomaviruses)

Human polyoma viruses such as BK virus and JC virus are well known pathogens in transplantation and are discussed elsewhere. In recent years three additional polyoma viruses have been described as potential pathogens in immunocompromised hosts. Like BK and JC, these viruses frequently cause asymptomatic primary infection in healthy patients and are capable of establishing latent infection which can be reactivated in the setting of immune suppression. KI and WU viruses (named for the institutions in which they were discovered, Karolinska Institutet and Washington University) have been isolated in children with acute respiratory symptoms including wheezing as well as in the setting of pneumonia [171, 172]. Respiratory infection has also been described in HIV-infected patients, in whom higher viral loads have been demonstrated in those with lower CD4 counts [173].

KI and WU polyomaviruses have been isolated in nasopharyngeal, sputum, and bronchoalveolar lavage specimens in hematopoietic stem cell and solid organ transplant recipients [174, 175]. These viruses have also been detected in transbronchial biopsy specimens in lung transplant recipients, who in many cases were asymptomatic. Coinfection with other viral and bacterial pathogens has been reported. RT-PCR results should be interpreted with caution in transplant recipients, in whom severe infection has not been described to date. There are no available data on the role of decreasing immunosuppressive therapy or the use of antiviral agents in the development or treatment of infection with KI and WU polyomaviruses.

Merkel cell carcinoma is a neuroendocrine malignancy of the skin which is most common in immunocompromised hosts including transplant recipients [173, 176]. Over 80% of these tumors contain a polyoma virus named Merkel Cell polyomavirus (MCPyV); virus has also been found in respiratory secretions in asymptomatic transplant recipients. Further study of each of these polyomaviruses is ongoing in the transplant population.

49.13 Rotavirus

Rotavirus, the most common cause of enteritis worldwide and a common pathogen in healthy children under the age of 3, has become increasingly recognized as a pathogen in pediatric and adult recipients of solid organ transplants [177]. Epidemics have occurred through fecal–oral transmission, primarily in the winter and spring. Affected patients present with watery diarrhea, nausea, vomiting, abdominal pain, and, in some cases, gastrointestinal bleeding from colonic ulcers. Infection may be diagnosed by antigen detection in stool specimens using ELISA, latex agglutination, or quantitative PCR. Infection is generally self-limited with weaning immunosuppression during the acute phase of illness. There are no published data on antiviral activity against rotavirus; treatment remains symptomatic.

Rotavirus has been associated with a high risk of acute cell-mediated rejection in intestinal transplant recipients, which has been proposed to be related to poor absorption of immunosuppressive agents in the setting of vomiting and diarrhea, as well as immune reactivation of gastrointestinal tract-associated lymphocytes in the setting of infection [178]. In HSCT recipients, rotavirus infection may be difficult to differentiate clinically and histopathologically from GVHD.

In 1998, a live, oral, tetravalent rhesus–human reassortment rotavirus vaccine (RotaShield, Wyeth-Ayerst Laboratories, St. David, PA) was licensed and recommended for routine immunization of infants in the USA; it was voluntarily withdrawn from the market in 1999 due to its association with intestinal intussusception noted in postmarketing surveillance [179–181]. Two additional Rotavirus vaccines have been studied (RotaTeq, Merck & Company, Whitehouse Station, NJ; Rotarix, GlaxoSmithKline Biologicals, Rixensart, Belgium). Both vaccines are oral and contain live virus, and are thus contraindicated in highly immunocompromised patients. Fecal virus shedding has been noted with both vaccines, with transmission of vaccine-associated virus to household members noted with Rotarix [181].

Current vaccination guidelines in immunocompromised hosts recommend that HSCT and solid organ transplant recipients not receive this live virus vaccine. Household contacts of patients with immune deficiency may be vaccinated, but the transplant recipient should not change diapers for 4 weeks after vaccination, the usual duration of viral shedding in stool [182].

49.14 West Nile Virus

West Nile virus (WNV) was initially isolated from a febrile patient in the West Nile Province in Uganda in the 1930s and has been enzootic in Africa, Asia, the Middle East, and parts of the Mediterranean and Europe, causing asymptomatic disease

or a self-limited febrile flu-like illness [183]. This flavivirus was first detected in the northeastern USA in 1999 and has caused outbreaks of infection in the late summer and early fall throughout the USA since then [184, 185]. Birds are the primary reservoir of infection. Mosquitoes acquire lifelong infection after biting viremic birds, spreading infection from their salivary glands to other species, including humans, with a subsequent bite. In human infection, the incubation period is 2–14 days [186]. While approximately 80% of infections are asymptomatic, 20% of patients develop West Nile fever, characterized by fever, malaise, anorexia, nausea, myalgias, headache, and occasionally lymphadenopathy [187]. One in 150 symptomatic patients develops meningitis and/or encephalitis [188]. Meningitis presents with photophobia, phonophobia, meningismus, and hyperreflexia; CSF analysis reveals a lymphocytic pleocytosis (<500 leukocytes/mm^3, glucose usually normal). Patients with encephalitis develop altered mental status, cranial nerve palsies, seizures, and movement disorders. A minority of patients develop rapid asymmetric weakness that may progress to flaccid paralysis mimicking poliomyelitis, associated with hyporeflexia or areflexia [184, 189, 190]. Acute neuromuscular respiratory failure may develop, which carries a mortality rate of more than 50% [188]. Hemorrhagic fever characteristic of other flaviviruses has also been described [191]. The presence of severe weakness and hyporeflexia in a patient with meningoencephalitis should raise the suspicion of WNV infection. MRI may demonstrate meningeal or periventricular enhancement, sometimes mimicking ischemic changes [186].

Transmission of WNV via dialysis has been suggested [192], and transmission via blood transfusion and organ transplantation has been well documented [193–197]. In immunocompromised hosts, central nervous system involvement is common, although CSF pleocytosis may be minimal [198–200]. Community-acquired infection has been reported following solid organ transplantation, occurring 2 months to 10 years posttransplant [185, 199–202]. A study of WNV infection during an outbreak in Toronto noted that liver, kidney, and heart transplant recipients had 40 times the risk of symptomatic infection as normal hosts [203]. In all cases, the recipients had participated in outdoor activities without the use of insect repellant or other personal protective measures. Fever often preceded neurologic symptoms. A delayed serologic response was noted in the transplanted cohort in which infection carried a mortality rate of 25%, versus 9% in the general population. In a Colorado outbreak in 2003, 11 transplant recipients (4 kidney, 2 liver, 2 kidney/pancreas, 1 lung, and 2 HSCT) developed infection requiring hospitalization [204]. Ten (91%) developed meningoencephalitis, one developed acute flaccid paralysis without encephalitis, and three patients had meningoencephalitis and paralysis. Two patients died (18% mortality), and three suffered significant neurologic sequelae. It appears that transplant recipients are more likely to develop meningoencephalitis in the setting of acute West Nile virus infection than immunocompetent hosts, perhaps with a higher mortality rate. Prolonged infection can also occur [205].

Several cases of WNV infection have been reported in HSCT recipients [206, 207]. Infection occurred 3–5 months posttransplant in the most well-described cases, after engraftment but while on calcineurin inhibitor-based prophylaxis or treatment of chronic graft-versus-host disease. Fever, lethargy, progressive bilateral extremity weakness, and hyporeflexia or areflexia were present. CSF contained 0–6 white blood cells/μL; IgG and IgM were negative in CSF and blood in most cases. Diagnosis of WNV infection was made by PCR performed on serum and CSF. All of the described patients died.

Diagnosis of WNV infection in immunocompetent hosts may be made serologically or via RT-PCR. An IgM antibody capture assay is available and becomes positive in CSF 3–5 days after onset of symptoms in nonimmunosuppressed hosts [202, 207], before serum antibody develops; CSF IgG appears approximately 5 days later. Antibody presence may be confirmed with viral neutralization studies. IgM antibodies may persist in serum for up to 12 months after infection resolution, and IgG may persist for years. As in the hematopoietic stem cell recipients noted above, immunocompromised patients demonstrate delayed seroconversion, making diagnosis of acute infection difficult at times. Nucleic acid testing in plasma and/or CSF is the most useful diagnostic test in this setting [208].

There are no antiviral agents that have proven efficacy in the treatment of WNV infection. Ribavirin possesses in vitro activity but demonstrates poor clinical efficacy [186, 209]. IVIg with high titers of anti-WNV antibodies (e.g., from Israel, where infection is endemic) has demonstrated significant clinical benefits in animal models, although antibody titers are low in immune globulin derived from the US donors, which have proven ineffective in treating acute infection [184, 210, 211]. A report of successful treatment of donor-transmitted WNV infection in a liver transplant recipient by reducing immunosuppression and administering plasma from seropositive blood donors has been published [212]. Overall case fatality rates of infection with WNV are 4–20% [189, 192], with significantly higher rates in transplant recipients.

Unlike the case in other neuroinvasive viral infections, the severity of initial clinical presentation does not predict the prognosis of WNV infection [187, 190, 213]. Survivors frequently suffer from prolonged fatigue, myalgias, cognitive deficits, memory loss, and tremors. Parkinsonism, excessive somnolence, and postural instability are reported. Phase I trials of a vaccine have been promising [214]. Transplant recipients should be educated about the transmission of West Nile virus and urged to remove any stagnant water collections and to use insect repellant when outdoors at dusk during the later summer and fall in order to prevent infection.

49.15 Conclusion

Viruses remain the most significant and elusive pathogens infecting patients following solid organ and hematopoietic stem cell transplantation. The days of "it's just a virus" are clearly behind us, as immunosuppression has changed, post-transplant longevity is increasing, and molecular diagnostic methods have dramatically improved [215]. Serology may be of limited value in immunocompromised hosts in the diagnosis of acute infection as well as in detecting reactivation of latent infections. Multiplex, quantitative real-time PCR assays are now available to detect multiple viruses, including panels of PCRs for detection of respiratory viruses and CNS pathogens [216, 217]. These sensitive techniques are being evaluated carefully in transplant populations for their specificity and for their potential utility as markers of early infection with surveillance monitoring. The impact of community-acquired respiratory viral infections on the development of acute rejection and bronchiolitis obliterans in lung transplantation appears to be significant and warrants further study [218, 219]. Continued vigilance in detecting emerging viral infections and continued study of potential antiviral therapies in the transplant population will likely improve patient survival.

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50 Travel Medicine, Vaccines, and Transplant Tourism

Camille Nelson Kotton

50.1 Introduction

As their overall health improves after transplant, transplant recipients may wish to experience foreign travel (including visiting friends and relatives). Such travel may entail exposures to endemic and sometimes unexpected pathogens. Transplant recipients are more likely to have complications from travel-related infections, are less likely to develop protection from vaccines, and are at higher risk for having drug interactions when they take new medications [1]. In addition, active infections and vaccines are immunomodulatory and could potentially impact immunologic tolerance. This review will summarize the medical literature regarding travel medicine and travel-related vaccines in the adult transplant recipient population. "Transplant tourism" (involving travel of either the organ donor or recipient strictly for purposes of organ transplantation) will also be covered here.

A travel medicine specialist familiar with their immunocompromised state and medications should see transplant recipients who wish to travel. Optimal care of this vulnerable population should include up-to-date, comprehensive advice and education on travel medicine, and various methods of protection. Travel health specialists for complex patients should confer with the traveler's other physicians (i.e., transplant physicians) as needed to develop an appropriate plan. Three surveys of transplant centers found insufficient rates of pre-travel counseling and intervention, and significant rates of illness in transplant recipients during foreign travel. In a survey of 267 solid organ transplant (SOT) recipients at the University of Toronto, 95 (36%) indicated that they had traveled outside Canada and the USA; 66% of travelers sought pre-travel advice, primarily from their transplant physician. In general, many of the recommended preventative measures were overlooked: 63% traveled to areas where hepatitis A is endemic, but only 5% had received hepatitis A immunization; 50% traveled to dengue- and malaria-endemic areas, but only 25% adhered to mosquito prevention measures; and 10% reported behaviors that exposed them to blood or body fluids [2]. A review at the Mayo Clinic, Rochester, Minnesota found that of 1130 solid organ transplant recipients, 303 (27%) reported travel outside of the USA or Canada after their transplant; 49 solid organ transplant recipients (16%) traveled to destinations at higher risk for infectious diseases. Travelers to these destinations were more likely to be male (73% vs. 54% of low-infection risk travelers, $P=0.018$) or born outside the USA or Canada (29% vs. 6% $P<0.0001$) [3]. Liver recipients were more likely to travel than other organ recipients. 96% of travelers reported that they did not seek specific pre-travel healthcare before their trip. 24 travelers (8%) required medical attention because of illness; illness was more likely among travelers to high-infection risk (18%) than low-risk (6%) destinations, $P=0.004$. Another cross-sectional, descriptive study of 290 Dutch kidney transplant recipients evaluated their travel health knowledge, attitudes, and practices while staying abroad. 34% had traveled outside Western Europe and Northern America; 22% of these travelers did not seek pre-travel health advice and 29% were ill during their most recent journey [4]. Transplant physicians were most frequently consulted for pre-travel advice (53%). Four of seventeen ill recipients (24%) were hospitalized, reflecting the high morbidity of travel-related disease in transplant recipients.

Optimizing the timing of travel may limit complications. Transplant recipients more than 2 years post-HSCT and who are not on immunosuppressive drugs and without graft-versus-host disease, or solid organ transplant recipients more than 1 year after transplant without heavy doses of immunosuppression or recent treatment for rejection are less immunocompromised and could be better able to face the risks associated with travel [5]. The spectrum and details of immunocompromise seen in transplant recipients are expansive [5, 6]. Those who also have AIDS with low CD4 counts, active leukemia or lymphoma, generalized malignancy, aplastic anemia, graft-versus-host disease, congenital immunodeficiency, or those who have received recent radiation therapy, treatment for rejection after SOT, or significantly immunosuppressive

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medications. Patients with chronic hepatic disease, chronic renal disease, diabetes, asplenia, and nutritional deficiencies are generally considered moderately immunocompromised, depending on the details of their disease.

In general, many of the recommendations for vaccination are the same in immunocompromised and non-immunocompromised hosts, with a few exceptions [1, 7]. When possible, vaccination for travel after transplant should be started several months before the trip, to allow time for further serologic evaluation and/or possible additional boosters. Emergency travel may present a potentially high-risk situation in which passive immunization could be used, such as administration of intramuscular immunoglobulin (i.e., gamma globulin) to protect against hepatitis A virus and other infections. To optimize the immunologic response, immunocompromised hosts should be vaccinated during periods of no or low exogenous immunosuppression when possible, i.e., before solid organ transplant, or well after HSCT. Vaccination may be avoided in the initial 3–6 months after SOT or hematopoietic stem cell transplant (HSCT), since this is usually their period of highest immunosuppression [8–10] when vaccines are less likely to trigger a robust immune response, and also to avoid confusion with early graft dysfunction or rejection. Whether vaccinations could contribute to acute rejection or graft-versus-host disease has been an area of debate, although this seems unlikely based on the available data.

Measurement of antibody titers following immunization may sometimes be useful. In general, a fourfold increase in titer is often considered evidence of seroconversion, and titers above a certain levels are considered evidence of seroprotection; both of these concepts were derived from data in normal hosts. Transplant recipients are less likely to have a significant immunologic response, although partial protection may be useful. Immune responses to vaccination also wane more rapidly. Booster doses of vaccine are occasionally administered to those with lower or undetectable antibody titers, but such practices have not been subjected to rigorous trials nor evaluated for protective efficacy.

50.2 Routine Vaccines

Adults often miss standard vaccines [11] and transplant recipients are no exception [12]. Some physicians, perhaps concerned about causing harm, may elect to skip vaccination of this vulnerable population. Annual recommendations for routine adult vaccinations, including immunizations for immunocompromised individuals, are available through the Advisory Committee on Immunization Practices and the Centers for Disease Control and Prevention website; other recent publications also have helpful travel-related guidelines for immunocompromised hosts [1, 6, 13–19]. Table 50-1 includes information on both routine and travel-related vaccinations in immunocompromised hosts.

HSCT recipients lose immunologic memory of previous exposure to infectious agents and vaccines, and therefore need to be revaccinated. There are numerous collaborative guidelines for revaccination after HSCT [20–22]. Standard recommendations for revaccination after HSCT include diphtheria and tetanus toxoids, pertussis vaccine, *Haemophilus influenza* type B conjugate, 23-valent polysaccharide and 13-valent conjugate pneumococcal vaccine, inactivated influenza and polio vaccines, and live attenuated measles–mumps–rubella vaccine, as well as others [23]. Re-immunization protocols may vary among transplant centers but should be considered in all recipients.

Tetanus is rare in the industrialized world, where vaccination rates are quite good; it has a much higher prevalence in resource-poor regions, although is still rare among travelers. Tetanus boosters are routinely recommended for SOT and HSCT recipients and should be up-to-date before traveling. Diphtheria is common in resource-poor regions with 5–10% mortality among normal hosts despite therapy. A diphtheria antibody level of >0.1 IU/mL suggests adequate protection. Patients with a lower titer and those vaccinated more than 10 years prior to travel should be revaccinated before entering an area in which diphtheria is endemic or resurgent. For immunocompromised travelers entering high-risk areas, diphtheria antibody levels may be measured a month or more after vaccination. Acellular pertussis vaccine is included in the combination vaccine with tetanus and diphtheria called Tdap. This has not been studied in immunocompromised hosts thus far, but could be considered for use in the appropriate setting [7].

Vaccination against influenza should occur annually in most immunocompromised hosts [7, 24]. Vaccination may be delayed in those who underwent transplantation, treatment of rejection, or other profound immunosuppression in the past few months, balancing the risks of infection with the likelihood of developing an immune response. Given the year-round influenza activity in the tropics, it may be prudent to vaccinate all immunocompromised travelers to those areas if they were not vaccinated within the past year. Influenza immunity wanes and it is not known whether such travelers should be given booster vaccines prior to travel. Pneumococcal vaccine should be given to immunocompromised hosts [24], and it may be sensible to vaccinate before travel. Data suggests that immunity to pneumococcal vaccine wanes more rapidly in renal transplant recipients, and conjugate vaccine does not improve the durability of response when compared with the polysaccharide vaccine [25]. Current guidelines recommend the use of conjugate pneumococcal vaccine followed by the polysaccharide vaccine [24, 26].

Measles is a global illness, with approximately 30 million cases annually, resulting in approximately 750,000 deaths. Measles vaccination in the USA is usually performed with a trivalent live viral vaccine (measles–mumps–rubella [MMR] vaccine). Live vaccines are generally contraindicated in immunocompromised individuals [5, 7, 15, 24, 27, 28].

Table 50-1. Vaccination in transplant recipients

Vaccine	Recommendation
Routine vaccines	
Influenza-parenteral	Yearly
Influenza-intranasal[a]	Contraindicated in patients/family members
Pneumococcal polysaccharide & conjugate	Recommended, with boosters
Tetanus/diphtheria/pertussis	Recommended
Human papilloma virus	Recommended
MMR[a]	Contraindicated
Varicella[a]	Contraindicated
Varicella zoster[a]	Contraindicated
Vaccines for selected transplant recipient travelers when indicated by destination and/or circumstances	
Bacille Calmette-Guerin[a]	Contraindicated
Hepatitis A	Recommended when indicated
Hepatitis B	Recommended when indicated
Japanese encephalitis	Recommended when indicated
Meningococcal polysaccharide	Recommended when indicated
Meningococcal conjugate	Recommended when indicated
Polio (OPV)[a] (oral)	Contraindicated in patients/family members
Polio (IPV) (injectable)	Recommended when indicated
Rabies	Recommended when indicated
Salmonella typhi Ty21a[a] (oral)	Contraindicated
Typhim Vi (injectable)	Recommended when indicated
Yellow fever[a]	Contraindicated

Adapted from "Advising Travelers with Specific Needs: The Immunocompromised Traveler" in Centers for Disease Control's "Health Information for International Travel" [5], the "Advisory committee on immunization practices recommended immunization schedule for adults aged 19 years or older--United States, 2015" [24], "Travel medicine and transplant tourism in solid organ transplantation" [1], and the "2013 IDSA Clinical Practice Guideline for Vaccination of the Immunocompromised Host" [7].
[a]*Live, attenuated.*

Although some pediatric data suggests vaccination may be immunogenic and without complications [29, 30]. Prior to travel to endemic areas, documentation of serologic evidence of immunity against measles, mumps, rubella, and varicella should be considered in transplant recipients. Immune globulin should be considered for measles-susceptible, immunosuppressed travelers who travel to measles-endemic countries and are at risk for exposure [31]. In general, MMR could be given to patients at least 24 months after HSCT and not on immunosuppressive medications [7, 21, 22].

Immunization against hepatitis B before travel may be indicated for certain immunocompromised hosts, including those living in endemic areas for extended periods, those with new sexual partners while traveling, or who are likely to need transfusions or medical procedures. Compared to the immune response following immunization pre-transplantation [15, 27], the efficacy of standard hepatitis B vaccination is reduced after transplantation (with response rates of 5–15%) [32]. In comparison, 20 liver transplant patients given extra doses of hepatitis B vaccine with one of two new adjuvants demonstrated a serologic response rate of 80% [33]. A group of 24 renal transplant patients who did not respond to intramuscular vaccine had an overall response rate of 63% to a series of eight intradermal vaccinations followed by an intramuscular vaccination [34]. For immunocompromised adults, some authorities recommend immunization with a vaccine containing 40 mcg of hepatitis B surface antigen (i.e., two 1 mL Engerix-B® vaccines, each containing 20 mcg, or a special formulation of Recombivax-HB®) given at one site, in a three- or four-dose schedule [35], although this regimen has been predominantly evaluated in dialysis patients. In a cohort of 292 recipients of unrelated or related allogeneic hematopoietic cell transplants given recombinant hepatitis B vaccine, 64% of patients seroconverted; in multivariate analyses, response was adversely affected by age older than 18 years and history of prior chronic graft-versus-host disease but not by donor type or by use of T-cell depletion, adoptive immunotherapy, or rituximab [36]. Interestingly, 89% of the non-responders mounted a threefold or greater rise in polio titers following 3 doses of inactivated poliovirus, thus the response to vaccination can be variable.

Varicella is less common in childhood in the tropics, especially in rural areas, and thus it is more common for an adult to have chickenpox than in the higher latitudes. Varicella (Varivax®) and varicella zoster (Zostavax®) vaccines are lower and higher doses, respectively, of the attenuated live Oka strain of varicella and in general their use should be deferred in transplant recipients until there is more data regarding their safety [24]. When possible, patients who are

seronegative for varicella should be vaccinated with two separate doses of varicella vaccine at least 1–3 months before undergoing exogenous immunosuppression, i.e., pre-solid organ transplant. There are several small studies in carefully selected pediatric SOT recipients given varicella vaccine, but similar data has not yet been shown in adult SOT recipients. One pilot study of 9 autologous HSCT recipients who were seropositive for varicella and who were vaccinated 3–4 months after HSCT with the Oka strain demonstrated a boost in varicella-specific cellular immunity as measured by lymphocyte proliferation, without significant systemic side effects [37]. One of the nine subjects developed varicella zoster at 3 months after vaccination. Heat-killed varicella vaccine was shown to be effective in HSCT recipients when given before and during the first 90 days after transplant but is not available outside of research settings [38].

50.3 Travel-Associated Infections and Recommended Immunizations

50.3.1 Hepatitis A

The risk of hepatitis A in non-immune travelers in resource-poor regions has been estimated to be 1 in 1000 per week for those on a usual tourist route, and 1 in 200 for those on more adventuresome travel [39]; a recent Swiss study showed much lower rates, with an actual incidence of hepatitis A in travelers to countries of high or intermediate risk of transmission of 3–11 per 100,000 person-months abroad for all travelers [40]. Hepatitis A could be a devastating illness in immunocompromised hosts. Pooled immunoglobulins, given as intramuscular gamma globulin, are 85–90% effective at protecting against hepatitis A infection, although this effect only lasts for 3–6 months (dependent on dose given). Some transplant recipients with hypogammaglobulinemia are given routine immunoglobulin repletion with intravenous immunoglobulin (IVIG); this dose is much higher (0.66 mL/kg, delivering at least ~100 mg/kg immunoglobulin) than the dose used for gamma globulin (0.02 mL/kg, the dose recommended for 3 months protection against Hep A, which delivers ~3 mg/kg immunoglobulin) and such patients would not need additional antibody protection. The preparations are similar, although IVIG has had immune aggregates removed such that it can be given safely intravascularly.

Hepatitis A vaccine is less effective in solid organ transplant recipients. In a study of 37 hepatitis A seronegative liver transplant recipients who were given hepatitis A vaccine 6 months apart, only 8% had seroconverted at 1 month following vaccination, and only 26% at 7 months (1 month after the second vaccination) [41]. In another study, zero of eight liver transplant recipients responded to the two doses of vaccine given 2 months apart [42]. In a third trial, liver and renal transplant recipients (39 in each group) received 2 doses of hepatitis A vaccine 6 months apart [43]; response after the primary dose occurred in 41% of the liver transplant patients and 24% of the renal transplant patients, while after the second dose, the respective conversion rates were 97 and 72%. A recent study of kidney transplant recipients showed a seroconversion rate of only 27% after two doses of vaccine [44]. Discrepancies between studies may be explained by differences in patient selection, severity of liver disease, immunosuppressive medications, and type of vaccine used. Importantly, organ transplant recipients have a more rapid antibody decline than controls: 2 years after vaccination, only 59% of liver transplant and 26% of renal transplant recipients who had seroconverted retained protective titers [45], in contrast to mathematical models of vaccination in normal hosts which predict antibodies to persist for at least 20–25 years [46]. Hepatitis A vaccine has not been well studied in the HSCT population, although a recent publication showed a seroconversion rate of 77% [47].

Overall, hepatitis A vaccine among transplant recipients results in attenuated rates of immunologic response and shortened durability. Use of higher or three or more doses of hepatitis A vaccine has not been studied in the immunocompromised population. If there is enough time before travel, it may be useful to vaccinate SOT recipient travelers with two doses of hepatitis A vaccine 6–12 months apart when the transplant recipients are at least a year after transplantation and are on a modest dose immunosuppressive regimen; titers could be checked to document seroconversion. SOT recipients who do not have adequate time before travel or do not respond to immunization should be given intramuscular immunoglobulin prior to travel [5].

50.3.2 Salmonella enterica serovar Typhi

An estimated 21 million cases and 222,000 typhoid-related deaths occur annually worldwide, according to the WHO (http://www.who.int/immunization/diseases/typhoid/en/). Approximately 400 cases of typhoid fever are reported in the USA each year, with 90% related to international travel, of which >75% involved India, Bangladesh, or Pakistan [48]. Severe complications can occur in immunocompromised individuals during infection with *Salmonella enterica* serovar Typhi and they should be immunized against typhoid prior to travel to endemic areas. There are currently two vaccines commonly available: the injectable polysaccharide vaccine (Typhim Vi®, Aventis Pasteur SA), and the oral live, attenuated vaccine Ty21a (Vivotif®, Berna). The live oral typhoid vaccine has not been shown to cause disseminated disease; however, for theoretical reasons, the inactive parenteral vaccine should preferentially be administered to immunocompromised individuals. While data are minimal in immunocompromised hosts, the immune response in immunocompromised hosts to either typhoid vaccine is usually

poor. A study of HIV positive patients vaccinated with the injectable polysaccharide vaccine found lower levels of antibody against *Salmonella enterica* serovar Typhi in vaccinated HIV-infected individuals compared with vaccinated healthy controls; the antibody responses in patients with CD4 cell counts of <200 cells/mm^3 were significantly lower compared with patients with ≥200 cells/mm^3, with geometric mean titers of antibodies to the *Salmonella* Vi antigen of 4 versus 35 arbitrary units (versus 106 in healthy volunteers), suggesting that HIV-infected individuals, even after vaccination, are more susceptible to an infection with *Salmonella typhi* when traveling to countries with a high incidence of typhoid fever [49]. Typhoid vaccination has not been well studied in HSCT and SOT recipients. As a relatively well-tolerated vaccine in general [50], and given the significant morbidity and mortality with typhoid fever, it may be prudent to vaccinate these populations with the injectable vaccine when they travel to endemic areas [48].

50.3.3 Polio

Poliomyelitis caused by wild-type poliovirus has been eradicated from the Western hemisphere; wild-type virus exists in sub-Saharan Africa and South Asia. Outbreaks of vaccine-associated poliomyelitis occasionally occur, due to neurovirulent reversion of live attenuated poliovirus from the oral polio vaccine. Vaccine-associated outbreaks of poliomyelitis have recently occurred in Hispaniola (Haiti and the Dominican Republic), the Philippines, Madagascar, and Cape Verde. Worldwide, two forms of the polio vaccine are available: the orally administered, live, attenuated virus (OPV or Sabin), and the injected inactivated poliovirus vaccine (IPV or Salk). Since attenuated vaccine strain polioviruses may spread through fecal–oral contact, transplant recipients and household contacts of immunocompromised individuals should not receive OPV. OPV is no longer distributed in the USA and Canada. Travelers should have received a primary series of polio vaccine during childhood and at least one booster as an adult. Some authorities recommend booster immunization if more than 10 years have elapsed since administration of the last polio vaccine, especially for individuals traveling to areas of the world with a polio outbreak or with circulating wild-type polio viruses.

The longevity of the response to revaccination with poliovirus after allogeneic stem cell transplant was studied in 134 patients who were given three doses of trivalent inactivated polio vaccine starting 12 months after HSCT and who survived at least 5 years after vaccination with a mean follow-up of 8 years (range, 1–19 years) [51]. 21 (15.6%) patients became seronegative to at least one of the poliovirus serotypes during follow-up; in multivariate analysis, the only risk factor for loss of immunity was younger patient age, and there was a strong trend for patients with chronic graft-versus-host disease to lose immunity more rapidly. All 14 patients given a booster dose of an inactivated poliovirus vaccine responded. Poliovirus immunity was thus shown to be retained long term after revaccination in most patients after allogeneic SCT. Response to vaccination after SOT has not been well studied, although should be considered in anyone traveling to an endemic regimen.

50.3.4 Meningococcus

Meningococcal disease has high case-fatality rates (5–15%). In the USA, a quadrivalent polysaccharide (*Neisseria meningitidis* A, C, Y, W-135) vaccine has traditionally been used; a similar protein conjugate vaccine was more recently approved for use. The meningococcal vaccine is indicated for individuals traveling to areas of the world with known outbreaks of invasive meningococcal disease, those traveling to the meningitis belt of sub-Saharan Africa (especially during the dry winter months of December through June), and for those traveling to Saudi Arabia for the Muslim pilgrimages of *hajj* or *umra*, where proof of vaccination is required. The response of solid organ transplant recipients to immunization with the quadrivalent protein meningococcal vaccine is poor, less than 50% were able to mount an immune response [52]. The majority of 44 patients who were given the vaccine 8 or 20 months after HSCT had significant immune responses to serogroups A and C; these responses were higher in individuals 20 months after transplantation than 8 months after transplantation and declined sharply over the first 6–12 months after vaccination suggesting revaccination should be considered for those at risks of exposure to meningococcal infection [53]. Data in pediatric patients after HSCT [54] suggests a good response to meningococcal serogroup C vaccination, while a less robust response was seen in pediatric oncology patients [55], especially those on chemotherapy or with lower B cell counts. Whether booster doses or combinations of polysaccharide and protein conjugate vaccinations are indicted is not clear for meningococcal vaccination; a study of pneumococcal polysaccharide and protein conjugate vaccinations in pediatric SOT did not show significant boosting [56]. A recently developed serogroup B vaccine may provide additional protection [57]. As transplant recipients are more likely to have significant morbidity and mortality from meningococcal disease, vaccination would seem prudent for those with potential exposure; safety and efficacy remain to be ascertained.

50.3.5 Yellow Fever

Yellow fever, a mosquito-borne viral hemorrhagic fever with a high case-fatality rate, occurs in tropical regions of South America and sub-Saharan Africa and kills an estimated 30,000 people every year. Case fatality may surpass 20% in normal hosts; no specific treatment exists. Yellow fever may

be a risk for travelers to endemic countries. The yellow fever vaccine contains a live attenuated viral strain and is distributed only through Department of Public Health-certified vaccination centers, including travel clinics and some county health departments.

As a general rule, the yellow fever vaccine should not be given to immunosuppressed individuals [5, 15, 58–60]. While a few mildly immunosuppressed travelers have tolerated the vaccine (including individuals with early HIV infection or a distant history of hematological malignancy not currently being treated with immunosuppressive agents) [61–64], complications including death have been reported in immunosuppressed individuals [5, 65]. Optimally, the immunocompromised traveler should avoid regions where yellow fever is endemic, or decrease risk by avoiding travel to those regions during peak season (i.e., January–March in Brazil, and July–October in rural West Africa) [5]. Transplant recipients should understand the risks of travel and minimize exposure to mosquitoes. When vaccination is deferred, a physician's letter stating contraindications to vaccination is acceptable to most governments. Such letters should bear the stamp of an official, approved yellow fever immunization center. Some countries may deny entry without immunization [5]. Family members of immunosuppressed persons may receive yellow fever vaccine.

When given prior to transplant, yellow fever vaccine has been shown to provide protection after SOT; in a series of 53 SOT recipients (including 29 kidney and 18 liver recipients) vaccinated prior to transplant, all but one (98%) had protective titers of neutralizing antibodies at a median duration of 3 years (range, 0.8–21 years) after transplant [66], suggesting adequate protection. However, the Advisory Committee on Immunization Practices 2015 guidelines, which state that a single primary dose of yellow fever vaccine provides long-lasting protection and is adequate lifetime protection for most travelers, recommend that HSCT recipients who had a dose of yellow fever vaccine pre-transplant and who are sufficiently immunocompetent to be safely vaccinated (generally, more than 2 years out from HSCT, without GVHD, and not on immunomodulatory therapy [5]) should be revaccinated before their next travel to yellow fever virus endemic regions, as prior immunity wanes significantly [67].

50.3.6 Rabies

Many travelers are at an increased risk of exposure to rabid animals while traveling. Long-term travelers, individuals expecting intense animal exposure, and individuals who plan to be far from medical care should be considered candidates for pre-travel immunization against rabies. Since transplant recipients may not mount adequate antibody responses to the rabies vaccine (titers >0.5 IU/mL are considered adequate), some authorities recommend administration of human rabies immunoglobulin (HRIG) after all at-risk exposures (normally, HRIG is only given to previously non-immunized individuals) [68]. Intradermal administration of rabies vaccines may result in variable immune responses even in immunocompetent individuals and is not recommended by most authorities. Data are minimal in SOT and HSCT recipients. One study of seven HIV+ patients with low CD4 T lymphocyte counts (<200 cells/uL) found poor neutralizing antibody responses to pre- and post-exposure rabies vaccination (even with doubling of the intradermal doses of cell-culture rabies vaccine); three HIV-infected patients with higher CD4 T lymphocyte counts (range 295–472 cell/uL) tended to have better antibody responses to post-exposure rabies vaccination [69]. Since transplant recipients may be less likely than others to participate in adventure travel or to spend long amounts of time away from civilization, vaccination should be considered in those with significant risks factors, and careful post-exposure prophylaxis is strongly advised.

50.3.7 Japanese Encephalitis

Japanese encephalitis (JE) may cause up to 10,000 deaths annually in Asia. Immunization against Japanese encephalitis should be considered for individuals with intense rural travel in areas of Asia endemic for JE, especially during periods of increased transmission [70]. The JE vaccine is an inactivated viral vaccine (although can be live attenuated, which should be avoided in transplant patients on active immunosuppression) and estimated to be 80–90% effective; hypersensitivity reactions in immunocompetent individuals occur in 0.6% of recipients and include generalized urticaria and/or angioedema, and less rarely neurologic adverse reactions including acute disseminated encephalomyelitis. The efficacy of the JE vaccine is largely unstudied in adult transplant recipients. In a trial in Thailand in pediatric HSCT recipients, 9/18 (50%) seroconverted at 3 months after a live attenuated single JE vaccination, although only 3/9 of these patients had sustained protective titers; 7/9 (78%) seroconverted at 3 months after a second JE vaccine injection, and all of these patients sustained protective titers at 12 months [71]. Another study showed that 7/8 pediatric living donor liver transplant recipients seroconverted when given inactivated JE vaccine >1 year after transplant [29].

Since this vaccine is more likely to elicit systemic reactive side effects, careful observation after administration with an eye to transplant graft function would be prudent.

50.3.8 Bacille Calmette-Guerin

Bacille Calmette-Guerin (BCG) is one of the most commonly administered vaccines in the world; a live, attenuated strain of *M. bovis*, it is used to prevent tuberculosis, especially in infants and children. BCG is rarely given in the travel medicine setting, and should be deferred in immunocompromised hosts, as they can develop a disseminated

infection. No specific prophylaxis other than infection control measures have been shown to be helpful in the immunocompromised population. IC hosts may wish to wear masks when in healthcare settings in areas endemic for tuberculosis. Pre- and post-travel tuberculosis skin tests with the purified protein derivative (PPD) or the newer gamma-interferon-based testing may be helpful, although they are more likely to be falsely negative in the immunocompromised population.

50.4 Vaccination of Close Contacts of Immunocompromised Hosts

Close contacts of transplant recipients could transmit some live, attenuated vaccine strains to the immunocompromised host. In general, certain live viral vaccines (including oral polio, nasal influenza, and smallpox vaccines) should be deferred from use in close contacts of immunocompromised hosts. Administration of other live vaccines such as measles, mumps, rubella, yellow fever, oral Salmonella, varicella (Varivax®) [72], and zoster (Zostavax®) vaccines are less likely to be transmitted and may be given to close contacts of immunocompromised hosts. If a rash develops with varicella vaccine, the immunocompromised host should avoid direct contact with the rash.

50.5 Non-Vaccine Preventable Illness and Immunocompromised Hosts

Diarrhea is the most common illness of travelers, affecting 10–60% of travelers to developing regions. Travelers' diarrhea may be life threatening to travelers with compromised immune systems. Dehydration may compromise renal function, and markedly increase toxicity of immunosuppressive agents such as tacrolimus. Complications of diarrhea may include bacteremia, metastatic seeding, and altered intestinal absorption (with concomitant alterations in the absorption of oral immunosuppressive medications). The oral cholera vaccine, available outside of the USA, has not been studied in immunocompromised hosts but has been safe in populations of healthy people and may provide protection. Prior to international travel, organ recipients should be instructed in appropriate food and water precautions. In general, SOT recipients should be cautioned to drink boiled or bottled water and other beverages, and to avoid food sold by street vendors and raw foods (except fruit and vegetables that can be peeled). If transplant recipients develop diarrhea for more than 1–2 days while traveling, especially with fever, vomiting, and/or bloody stools, they should consider seeking medical attention, and they should carry appropriate self-treatment such as ciprofloxacin or azithromycin. Due to microbial resistance, trimethoprim–sulfamethoxazole is generally ineffective against travelers' diarrhea. There are no data regarding the use of antimotility agent in transplant recipients with diarrhea, but such agents may serve to delay clearance of toxins from the gut. In the gastrointestinal tract, bismuth subsalicylate (i.e., Pepto-Bismol) is converted to salicylic acid and insoluble bismuth salts; transplant recipients with decreased renal function may be at higher risk for salicylate toxicity. Prophylaxis against bacterial traveler's diarrhea with daily antibiotics is rarely indicated and should only be considered for short-term use, after considering the risks of antibiotic resistance, *Clostridium difficile* colitis, potential for drug interactions and side effects.

Respiratory infections are the second most common infection affecting travelers [39]. Endemic fungal pulmonary infections, such as histoplasmosis and coccidioidomycosis in North America, and penicilliosis due to *Penicillium marneffei* infection in Southeast Asia, could be acquired during travel [73]. SOT recipients are at higher risk for invasive fungal infection, and should avoid activities such as spelunking and excavating, activities that have been associated with exposure to *Cryptococcus neoformans* or endemic fungi. The appropriate use of masks may be helpful. For those with significant exposure to tuberculosis, pre- and post-travel testing may be indicated. Interferon-gamma release assays, i.e., Quantiferon TB or T SPOT TB, have been shown to be more sensitive than tuberculosis skin testing in transplant recipients [74].

Travel to the tropics has been shown to result in higher rates of multidrug-resistant Enterobacteriaceae, with travel to these regions posing a risk factor for colonization with such organisms during the first 3 months after return, but not beyond [75]. A survey of travelers from the Netherlands found that 30% acquired extended-spectrum β-lactamase-producing Enterobacteriaceae during travel [76]. Such exposure could be a concern for transplant patients who are more vulnerable to infection.

Malaria and dengue fever are the most common arthropod-borne illnesses of travelers. Most cases of dengue fever are self-limited in the normal host; the risk for complications in transplant recipients is unknown. Malaria is a significant risk for all travelers to endemic areas. Prophylaxis against malaria should be based on the travel itinerary; the CDC Yellow Book provides country-specific guidelines. Transplant recipients should be instructed on ways to minimize insect bites, including use of repellents containing DEET (*N*,*N*-diethyl-3-methylbenzamide), bed nets, protective clothing, and permethrin-impregnated clothing.

Travelers to endemic regions may contract parasitic infections such as *Strongyloides stercoralis* infections, when larvae from contaminated soil penetrate skin or mucous membranes. Unlike other intestinal parasites, *Strongyloides* can replicate inside the human host, which allows the perpetuation of autoinfection; *Strongyloides* infection may persist for decades. *Strongyloides* infection can flourish in the setting of immunosuppression, resulting in hyperinfection.

Travelers should wear socks and shoes to avoid contact with this and other pathogens. Swimmer's itch (due to *Schistosoma* spp.), cryptosporidiosis, and other parasitic infections can be prevented by avoiding swims in non-chlorinated fresh water.

Transplant recipients have a markedly increased risk of skin cancer that correlates with the intensity of sun exposure, and it is important to recommend the use of hats, sunglasses, protective clothing (also useful for arthropod-borne infections), and sun protection lotions with ultraviolet A and B protection.

Travelers who rapidly ascend to altitude are at risk for altitude sickness. Acetazolamide accelerates acclimatization and decreases the risk of altitude sickness [77]; its use in organ transplant recipients is unstudied. Travelers to high attitude should be advised to avoid vigorous activities for the first few days at altitude. Acetazolamide should be offered to those travelers ascending rapidly to greater than 2500 m since there is at least a 15–25% risk of altitude sickness.

Drug interactions are of particular concern in transplant recipients, and they should be cautioned about using new medications that may be given by unknowledgeable practitioners or purchased "over the counter." Chloroquine can increase serum levels of cyclosporine and perhaps sirolimus and tacrolimus. Data are limited regarding other possible interactions between travel-associated drugs and immunosuppressive medications (Table 50-2). Short courses of ciprofloxacin or azithromycin for travelers' diarrhea seem unlikely to have a major impact on cyclosporine levels.

Acquisition of new virus should be avoided, either by safer sex practices, use of clean needles and syringes or avoidance of blood transfusions in foreign countries. Sterile needles and syringes may be given to a traveling transplant recipient with a physician's letter stating they are for medical use. Patients with end-stage renal disease, either prior to or after organ transplant, and who undergo hemodialysis in resource-limited countries, where suboptimal infection control policies pose a risk of exposure to blood-borne viruses, are at significant risk of acquiring new viral infections A number of cases of hepatitis C have been reported in Western travelers to the Indian subcontinent, Tenerife, Saudi Arabia, Singapore, and Slovakia [78, 79]. Such exposures may have an impact on policies in transplant centers regarding evaluation of those on the waiting list for solid organ transplant [80].

Educational topics for traveling transplant patients are covered in Table 50-3, and many of these points should be emphasized both during travel medicine visits and by the transplant clinicians discussing travel.

50.6 Transplant Tourism

Medical tourism has emerged as a global health care phenomenon, estimated at $60 billion worldwide in 2006 [81]. Transplant tourism, defined as travel with the intent of receiving or donating a transplanted organ, has grown tremendously in the past decade. While "emotionally related" transplants may be occurring in these regions, the majority is likely to be for payment to the donor, also known as "commercial transplants." A review of US national waiting list data identified 373 foreign transplants (173 directly noted; 200 from data validation); most (89.3%) were kidney transplants, and male sex, Asian race, resident and nonresident alien status, and college education were significantly and independently associated with foreign transplant in 35 countries, led by China, the Philippines, and India [82]. Numerous international transplant organizations, including The Transplantation Society and The International Society of Nephrology, have made major efforts to decrease such purchase and sale of organs on ethical grounds [83]. The practice of "transplant tourism" is relatively common and increasing, nonetheless, and transplant clinicians should be aware of the infectious disease risks [84, 85].

Table 50-2. Interactions between transplant and travel-related medications

	Calcineurin inhibitors (CNI)	Trimethoprim/sulfamethoxazole	Sirolimus, everolimus
Azithromycin	May ↑CNI levels		
Mefloquine	May ↑CNI levels	May increase risk of cardiac toxicity, QT prolongation, torsades de pointes or cardiac arrest	May ↑ sirolimus levels
Atovaquone-proguanil		May increase risk of proguanil of bone marrow toxicity	
Doxycycline	May ↑CNI levels		May ↑ sirolimus levels
Chloroquine	May ↑CNI levels	May increase risk of cardiac toxicity, QT prolongation, torsades de pointes or cardiac arrest	
Ciprofloxacin, levofloxacin	May increase risk of cardiac toxicity, QT prolongation, torsades de pointes or cardiac arrest		
Primaquine	May ↑CNI levels		
Sulfadoxine/pyrimethamine	May ↓CNI levels	May increase risk of bone marrow toxicity	
Acetazolamide	May ↑CNI levels		

Significant interactions of travel medicines and azathioprine, mycophenolate mofetil, and corticosteroids have not been reported; significant interactions of transplant medicines and diphenoxylate hydrochloride and atropine sulfate tablets or loperamide have not been reported; minimal data available. Adapted from MicroMedex® DrugReax® interactive drug interactions and Lexi-Comp OnlineTM interaction analysis.

TABLE 50-3. Educational topics for transplant recipients who wish to undergo travel

Timing of travel after transplant (>1 year for SOT, >2 years for allo-HSCT)
Destination and level of risk of infectious complications, availability of medical care for transplant patients
Clean food and water; administrations of antibiotics and hydration with significant diarrhea
Mosquito and insect precautions
Avoiding blood-borne infections from transfusions, injections, tattoos, and avoiding sexually transmitted diseases
Carry extra transplant medications, first aid kit
Sun protection
Drug interactions and risks of fake medications
Risk of *Vibrio* infections from ocean water (especially with liver disease)
Notify clinicians about recent travel if ill upon return

A meta-analysis comparing the risks of participating in transplant tourism by acquiring a kidney abroad to the risks associated with domestic kidney transplant demonstrated that transplant tourists are significantly more likely to contract cytomegalovirus, hepatitis B, HIV, post-transplantation diabetes mellitus, and wound infection than those receiving domestic kidney transplant, with lower patient- and graft-survival rates [86]. Many of these organ transplants are not recorded in databases, so the incidence of infection is unknown. The extent and quality of the pre-transplant evaluation of the donor and recipient is likely to be quite variable. When serologic evaluation is performed, the quality of the testing may be substandard. A significant number of foreign-born transplant recipients will return to their country of origin for organ transplants, and are at risk both for reactivation of latent infections as well as acquisition of new indigenous infections; knowledge of the specific infections existing in those regions, as outlined in a recent review [87], can direct further evaluation. Documentation and communication with the transplant center may be limited, and prophylaxis against infection may be imperfect [88].

Rates of hepatitis B, HIV, and other infections may be much higher in certain regions, including Asia. One review found new, transplant-related infections of HIV at rates of 4–6% and hepatitis B at rates of 2–12% in recipients who had undergone commercial transplants in foreign countries [88]. In a study comparing 540 patients who had received commercial renal transplants in India between 1978 and 1993 with 75 recipients of emotionally related renal transplants performed at two participating institutions in the Middle East, they found that graft-survival rates were similar, but that there was a higher incidence of human immune deficiency virus (HIV) infection (5% vs. 0%), and hepatitis B virus infection rate (8% vs. 1%) [89]. A case report from England documents de novo hepatitis B infection in a patient who received a renal transplant in India, with subsequent infection of 4 patients in England (due to breaks in infection control practices), including another renal transplant recipient and his wife [90].

Several institutions in more industrialized countries have described their experience with transplant tourism, and the subsequent risks of infection. A series from Turkey compared 115 patients who had been commercially transplanted in various countries (India (106 cases), Iraq (7 cases), and Iran (2 cases)) and subsequently seen at their center for post-transplant care between 1992 and 1999 with those with a living related transplant performed at their center [35]. The post-transplant course in the commercially transplanted recipients was more complicated, with infections caused by malaria (10 cases), invasive fungal infections and tuberculosis (5 cases each), and pneumonia due to various opportunistic pathogens. A survey of 16 patients in Australia who underwent commercial renal transplantation abroad found higher rates of infectious complications, including 2 who acquired hepatitis B at the time of the transplant and subsequently died from the infection [88]. A review of 10 patients who underwent evaluation for transplant in Minnesota and subsequently had transplants abroad (8 in Pakistan (all Somali origin), one in China (Chinese origin), and one in Iran (Iranian origin)) found that complications were primarily infectious, with six potentially life-threatening infections in four patients, including severe wound infection, *Acinetobacter* bacteremia/sepsis, central nervous system *Aspergillus* infection, severe urosepsis in 2, and CMV infection [91]. A survey of 18 patients who traveled from western Scotland to Pakistan from 2000 to 2007 for renal transplant found that overall, they did relatively well, with one case of malaria, and no cases of hepatitis B, hepatitis C, or HIV infection [92]. A review of 33 kidney transplant recipients who traveled abroad for transplant and returned to University of California, Los Angeles had post-transplantation outcomes compared with a matched cohort of patients who underwent transplantation at UCLA [93]. Most patients traveled to their region of ethnicity with the majority undergoing transplantation in China (44%), Iran (16%), and the Philippines (13%). Seventeen (52%) patients had infections, with nine requiring hospitalization. One patient lost her graft and subsequently died from complications related to donor-contracted hepatitis B. Almost all of these studies report reduced graft and patient survival, higher infectious complications, and higher rates of rejection.

When these transplant recipients return to transplant centers in industrialized countries, it is prudent to consider screening them for blood-borne pathogens, including HIV, HBV, and HCV, as well as bacteremias, urinary tract infections, and other endemic pathogens depending on their clinical course (malaria, tuberculosis, Chagas disease, etc.). Optimizing their post-transplant prophylaxis against infection and obtaining further information about their surgical procedure(s) and immunosuppression may also help optimize their care.

50.7 Conclusion

Every year there are more transplant recipients with an increasing variety of immunologic deficits. As their health improves, they may wish to travel more frequently. Research on vaccines and transplant recipients in recent years has been quite helpful in eliciting the potential immunogenicity and safety of various vaccines in this population. Hopefully within the next 5 years we will begin to understand more of the immunology in these hosts, which should allow for better vaccination. Improved vaccines, the ability to safely give adjuvants to boost immunogenicity, and more selective immunosuppression may allow for better protection of travelers in this vulnerable population.

In summary:

- Transplant recipients are increasing in number, as is the extent of global travel, thus this issue will continue to expand. Further studies are needed and will help guide clinical management. Prior to foreign travel, it is prudent to have transplant recipients seen by travel medicine specialists familiar with this complex and vulnerable population. Travel vaccines should be guided by the details of the travel in combination with details of the immunosuppressive regimen.
- Transplant recipients are more vulnerable to infection and are less likely to have a strong immunologic response to immunization. Vaccination either before undergoing immunosuppression, or optimizing the time of vaccination after immunosuppression, may help optimize the immunologic response.
- Routine immunization is important to consider and may have been overlooked or avoided in this population. Routine immunization should be considered before patients undergo solid organ or stem cell transplant. In addition, booster doses should be considered, especially after HSCT.
- Although not generally evidence based, additional or higher doses of certain vaccines may result in better protection, as has been demonstrated with hepatitis B vaccine in immunocompromised hosts.
- Immunoglobulin may provide protection against hepatitis A, measles, and other illnesses when the recipient is less likely to have an immunologic response, vaccination is contraindicated, or does not have enough time to develop protection.
- Evaluation of serologic response after vaccination may provide an index of seroprotection and may help guide the use of additional vaccinations. Serologic response is primarily a measure of humoral immunity and does not generally include information on cellular immunity. Even in situations where the antibody titers are low or undetectable, these subjects may be more protected than those that were never vaccinated (i.e., even minimal immunity may be better than none).

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Microbiome in Transplantation

Ying Taur

51.1 Introduction

The human microbiota refers to the collective of microorganisms inhabiting the human body, and the human microbiome refers to the aggregate of microbial genomes from that consortium. These complex populations harbor a vast amount of microbe-to-microbe diversity, but also vary greatly from person-to-person and body site-to-body site [1]. Close ties exist between these microbes and the human they inhabit, performing essential tasks that are mutually beneficial. Given the dependence of human hosts on these microbes for continued health, it may not be surprising that many human diseases have been found to be related to disturbances of the microbiome. Disorders with observed links to the microbiota span many medical disciplines, including *Clostridium difficile* infection, inflammatory bowel disease, obesity, type 2 diabetes, asthma, eczema, and autism [2–6]. The full extent by which the microbiome is linked to human health may not yet be fully realized.

Study of the human microbiota has been made more feasible through significant advances in molecular approaches which allow researchers to profile microbial communities in great detail. Previous approaches that relied on microbiologic culture provide a biased and incomplete view, consisting of a very small subset of microorganisms comprising the microbiota. DNA sequencing data can now be obtained from entire microbial communities simultaneously in parallel fashion, at a fraction of the cost compared with conventional sequencing methods [7]. These approaches have allowed for large scale endeavors such as the Human Microbiome Project, which seeks to comprehensively characterize and catalogue the human microbiome [1], but also smaller studies at the individual investigator level.

The earliest and most commonly used approach is to target and sequence the 16S rRNA gene, a conserved region which can be used to identify bacteria and differentiate to species level. Similarly, ITS1 and ITS2 regions have been used as targets for fungal identification [8]. Metagenomic studies, in which the entirety of DNA of an entire community is sequenced, can be accomplished using shotgun sequencing, in which millions of random genomic fragments are sampled from a microbial community. These methods are more expensive and require more sequencing depth compared with targeted sequencing of 16S or ITS1, but can provide data on functional content of microbes.

The data resulting from amplicon sequencing and whole metagenome sequencing can be analyzed using a variety of methods. Sequences can be matched to reference sequences to infer taxonomic classification or functional content, and/or grouped into clusters of similar sequence identity referred to as operational taxonomic units (OTUs). The relative abundance of various OTUs can be used to calculate estimates of overall diversity in the sample. Shotgun reads can be assembled to determine functionality by matching to functional databases [9].

In addition to DNA sequencing, advances in mass spectrometry methods have given rise to metabolomics, in which the complete set of small molecule metabolites in a particular environment are quantitatively measured. This technology has also been applied to microbiome studies, often complementing sequencing data and providing further understanding of microbial dynamics and its relationship to host.

In the setting of hematopoietic stem cell transplantation (HCT) and solid organ transplantation (SOT), the microbiome has been studied for clinical relevance, using the approaches described above, and has shown itself to be an exceedingly important factor in the outcomes of transplant recipients. This chapter will provide an overview of the structure and function of the human microbiota, including characteristics of its most important and notable members. With this foundation, the relevance of the microbiota in the transplantation setting will be reviewed.

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51.2 Overview of Human Microbiome

An adult human body is home to approximately 100 trillion bacteria, distributed over several body sites [10]. Each geographic body site represents a specific microbial niche, each with a distinctive composition and based on surrounding environmental factors [1]. The microbiome within the gastrointestinal tract is the most well-studied ecosystem, which has demonstrated numerous orchestrated interactions with the host, as well as the clearest links to human health. Moreover, the intestinal microbiota is the largest and houses the greatest variety of bacteria, many of which are not found in any other environment.

The host intestinal tract exerts influence and control over the composition of the microbiota by producing factors designed to resist pathogens and favor beneficial commensal bacteria, including antimicrobial peptides such as defensins [11]. Immune systems specific to the intestinal tract serve to resist translocation and systemic dissemination of bacteria into outside tissues; these include gut-associated lymphoid tissues, such as Peyer's patches, dendritic cells, and specific T- and B-cell subsets [11]. The mucus layer produced by Goblet cells also helps to provide further protection against dissemination. In turn, healthy commensal members of the intestinal microbiota help to modulate these host mechanisms, contributing to microbial stability within the gut lumen.

Under normal circumstances, the intestinal microbiota is largely comprised of anaerobic bacteria that are essentially non-pathogenic and serve a variety of functions that are beneficial to the human host, such as absorption and breakdown of nutrients, production of short chain fatty acids and vitamins, amino acid synthesis, detoxification of foreign substances, maintenance of mucosal wall integrity, and stimulation and development of host immunity [11, 12]. An important function of the healthy microbiota is to promote colonization resistance, which allows the microbial community to resist overgrowth or infection by pathobionts [13]. Many commensal organisms in the gut exhibit this behavior, suggesting a deep and long-lived relationship with their human hosts.

To gain an understanding of the structure and function of the human microbiome, an overview of the bacterial phylogeny of typical microbiome inhabitants is useful. Figure 51-1 shows a phylogenetic tree of common intestinal bacteria. At the phylum level, the four most abundant groups are Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria. Of these, Firmicutes and Bacteroidetes have the highest abundance; normally, at least 90% of all intestinal bacteria belong to one of these phyla in human adults. Actinobacteria and Proteobacteria are less abundant but almost always present in a stable gut microbiome. These four phyla are described briefly here.

51.2.1 Firmicutes

Firmicutes are the most abundant phylum in the gastrointestinal tract, typically occupying over half of the microbiota in healthy adults. Included are potential pathogens such as *C. difficile*, *Staphylococcus aureus*, *Streptococcus* spp., and *Enterococcus faecium*. Most members are Gram-positive, but in some cases can be Gram-negative, and metabolically can range from aerobic to obligately anaerobic.

Clostridia represent the largest class within Firmicutes, which house a large number of Gram-positive spore-forming obligate anaerobes. Phylogenetic studies have shown great heterogeneity in these bacteria, and have shown that older classifications of these bacteria were flawed, bringing to light several misclassifications, and at times necessitating revisions of taxonomic classification within these bacteria. One phylogeny-based grouping approach divides Clostridia into approximately 20 clusters, numbered I through XIX, and can be used to differentiate and describe these microbes [14]. These groups demonstrate several lines of divergence within the evolutionary phylogeny of Clostridia, which form the basis of heterogeneity among these bacteria. For the most part, potentially pathogenic Clostridia seem to largely reside exclusively within two clusters: Cluster I, which includes toxin-producing bacteria such as *Clostridium perfringens*, and Cluster XI, which includes *C. difficile* and *Clostridium sordellii*. The remaining clusters, however, constitute a significant portion of the healthy commensal microbiota. In particular, Clostridium clusters IV and XIVa, whose members span the families Ruminococcaceae and Lachnospiraceae, are most abundant and appear to be critically important members. These groups perform beneficial functions such as degradation of complex sugars. *Faecalibacterium prausnitzii* of cluster IV, for example, has been well studied for its ability to degrade sugars in its various forms to short chain fatty acids such as butyrate [15]. Furthermore, these bacteria can modulate inflammation through several mechanisms, such as induction of regulatory T-cells [16, 17]. Lachnospiraceae, a family which includes *Clostridium scindens*, have been shown to have suppressive effects on *C. difficile* infection [18, 19].

Non-clostridial members of Firmicutes include the genera *Streptococcus*, *Enterococcus*, and *Staphylococcus*, which are known for their potential for pathogenicity and are responsible for a great many human infections. Antibiotic resistant forms such as MRSA and VRE are particularly difficult challenges in the clinical setting. Under normal circumstances, these exist in relatively low abundances, and may even participate in breakdown of nutrients [20]. However, expansion and domination can occur in the setting of microbiota perturbation, leading to several important infections, such as bloodstream infection during HCT [21].

51.2.2 Bacteroidetes

Bacteroidetes are Gram-negative anaerobic bacteria that typically inhabit the gut microbiota in high abundance, and include members such as *Bacteroides* spp. and *Prevotella* spp. These bacteria are highly suited for the gut; some members, such as *Bacteroides fragilis*, can only be found exclusively in the gastrointestinal tract of humans. This is evidenced by the fact

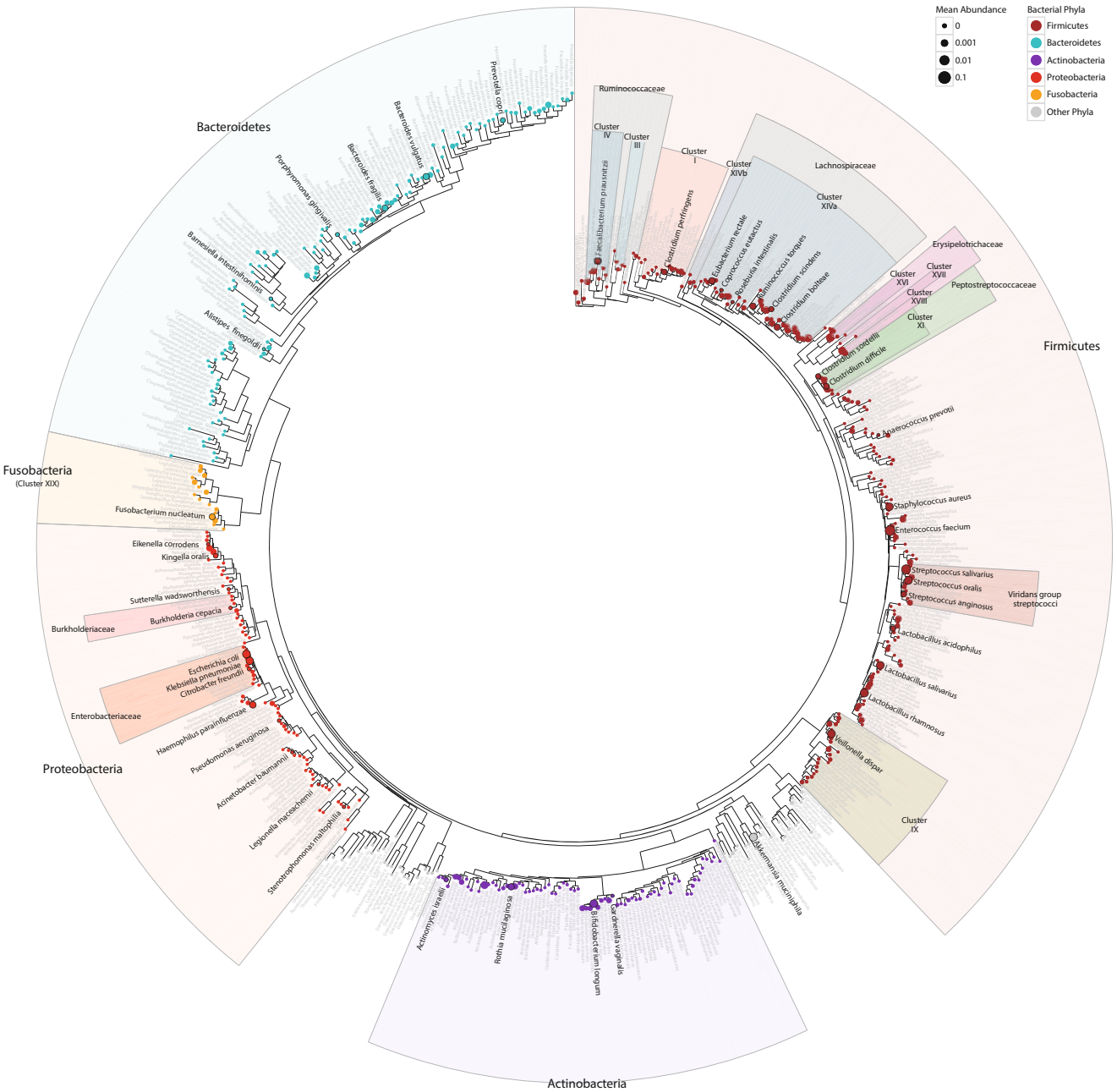

FIGURE 51-1. Phylogenetic tree of most common bacteria found in the human microbiome. *Colored circles* represent single bacterial species, size reflects the approximate mean intestinal abundance observed in HCT recipients. Species labels are listed in *black* for notable or particularly abundant members. *Outer labels* represent the bacterial classification at phylum level; *middle labels* show several bacterial groups at the family level. Clostridium clusters and viridans group streptococci are also labeled; note that these are not true taxonomic classifications.

that Bacteroidetes are specifically selected for colonization by host immunity; stable residence in the gastrointestinal tract is dependent on host–microbe interactions such as toll like receptors [11, 22]. Established Bacteroidetes, in turn, induce the homeostasis of regulatory T-cells [23], making them the most stable.

Bacteroidetes perform essential metabolic conversions important for the host, perhaps most notably the fermentation of complex carbohydrates, with the resulting production of short chain fatty acids, which are absorbed and utilized by the host and other bacteria for energy and maintenance of gut health [24, 25]. Bacteroidetes offer protection against *C. difficile* infection: individuals with recurrent CDI were deficient in Bacteroidetes, and replenishment of Bacteroidetes with fecal microbiota transplantation resulted in durable cure [26, 27].

51.2.3 Proteobacteria

The Proteobacteria phylum is perhaps best known for containing many well-known Gram-negative intestinal pathogens, such as Enterobacteriaceae, a family of bacteria that includes *Escherichia coli* and *Klebsiella pneumoniae*, and *Citrobacter freundii*. Other known pathogens within Proteobacteria include *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Stenotrophomonas maltophilia*. The predilection for various forms of antibiotic resistance in these bacteria has given rise to some of the most difficult-to-treat infections in humans.

Despite its pathogenic members and wide genetic variety observed in culture, Proteobacteria represent only approximately 1 % of a stable gut microbiota, under normal circumstances [28]. However, under aberrant conditions, potentially pathogenic members can bloom, increasing in abundance to become dominant in the microbial space, which can enable certain Proteobacteria to become pathogenic.

51.2.4 Actinobacteria

The Actinobacteria phylum is a group of Gram-positive bacteria that colonize several body sites including gastrointestinal tract and skin. Familiar members of this phylum include *Bifidobacterium* spp., *Propionibacterium acnes*, *Corynebacterium* spp., *Gardnerella vaginalis*, *Actinomyces israelii*, *Nocardia asteroides*, and *Mycobacterium tuberculosis*. Though some are implicated in infections, Actinobacteria in general are highly adapted to stable colonization within humans [29]. In the intestinal tract, members such as *Bifidobacterium* spp. appear early in gut colonization, occupying a significant portion of the intestinal microbiota during infancy. Given their observed beneficial impact on human health, some are formulated in probiotics. These bacteria perform a variety of metabolic functions that are beneficial to the host, including fermentation of simple and complex sugars, deconjugation of primary bile acids to secondary bile acids, and protein degradation [28].

Other functions of Actinobacteria may be relevant in HCT; these bacteria also are known to promote mucosal barrier integrity, enhance secretory IgA, induce regulatory T-cells, and promote colonization resistance against potential pathogens such as Enterobacteriaceae and *C. difficile* [17, 30–34]. These benefits may have been reflected in preliminary observations in HCT; recipients with greater abundances of Actinobacteria were observed to have improved outcome in one study [35].

51.3 The Microbiome in Hematopoietic Stem Cell Transplantation (HCT)

In the transplant setting, allogeneic HCT recipients have been the most extensively studied for clinical ties to the microbiome, particularly in the intestinal tract. This is in part related to the fact that the gut is seemingly at the center of many transplant-related complications. During HCT, recipients are subjected to combination of chemotherapy, total body irradiation, and broad-spectrum antibiotics, which significantly impact host immunity, damage intestinal epithelial linings, and disrupt previously stable microbe communities. These simultaneous insults help explain the frequency of complications such as bloodstream infections, *C. difficile* infection, and graft-versus-host disease.

The importance of the intestinal microbiota in HCT is supported by the observation that patients with low bacterial diversity at the time of stem cell engraftment are significantly more likely to die over the next 3 years compared to those with higher diversity microbial communities [35]. One prospective study showed an association between microbial diversity and transplant-related mortality that was independent of other established risk factors such as disease status, pre-transplant comorbidity, and myeloablative intensity. However, the specific details and potential mechanism of this observed link are still unknown.

Disruption of the intestinal microbiota primarily occurs during the early phase of HCT (Figure 51-2), shortly after administration of conditioning chemotherapy and radiation, along with antibiotics, given either prophylactically or as treatment [21]. The degree by which the microbiome is affected during this period varies greatly from person-to-person, even among recipients undergoing treatment of similar intensity. Some of this heterogeneity can be explained by antibiotic administration. Ultimately however, the factors influencing the degree to which a recipient's microbes can resist the disruption of microbial diversity during transplant are still relatively unresolved. The consequences arising from the perturbation of the intestinal microbiota can be seen in several infectious and non-infectious complications of allogeneic HCT; some are discussed below.

51.3.1 Vancomycin-Resistant Enterococcus (VRE) Bloodstream Infections

VRE bloodstream infections have increasingly become a major cause of bloodstream infection in patients with severe or prolonged absolute neutropenia, such as in the pre-engraftment phase of HCT [36–40]. VRE has become the most common pre-engraftment bloodstream infection at many transplant centers [39].

Numerous studies indicate that antibiotic administration, particularly treatments with activity against anaerobic bacteria, is the primary driver of VRE colonization [41–43]. In this setting, VRE has a remarkable ability to densely populate the small and large bowel, quickly occupying over 98 % of the intestinal microbiota niche [44].

Intestinal domination of the microbiota by VRE is frequently observed in HCT, and has the notable consequence of systemic infection during pre-engraftment neutropenia. Longitudinal study of the intestinal microbiota in alloge-

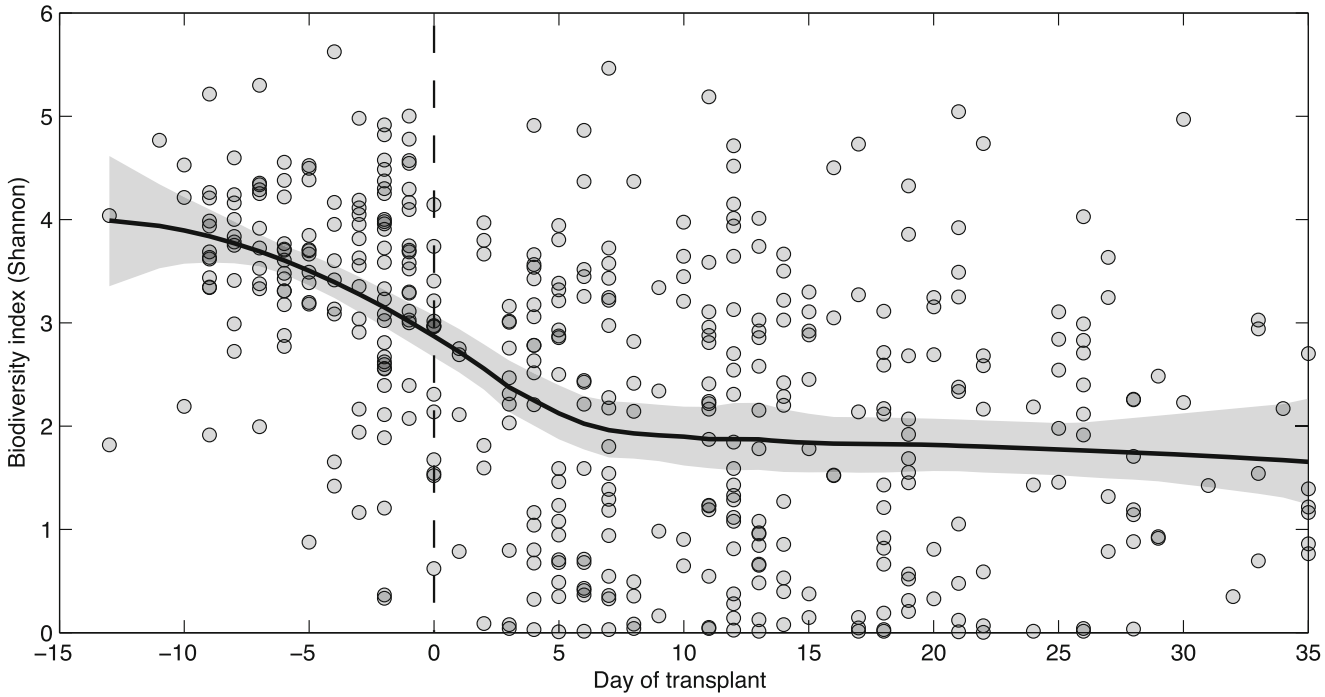

FIGURE 51-2. Changes in microbial diversity within the intestinal tract during allogeneic HCT. Diversity of intestinal bacteria, quantified by Shannon index, is shown for 439 fecal specimens (*circles*) collected serially from 94 patients over the course of transplantation. Intestinal diversity decreases during this time (moving average shown as *solid black line* with 95% confidence interval). Taur Y, Xavier JB, Lipuma L, et al., Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation, Clin Infect Dis, 2012, 55(7):905–14, by permission of Oxford University Press.

neic HCT has shown that VRE expansion precedes VRE bloodstream infection [21]. This expansion, in turn, is frequently preceded by prior administration with metronidazole, a drug with potent anti-anaerobic activity. This indicates that antibiotic disruption of healthy anaerobic microbiota (e.g., Bacteroidetes, Clostridium clusters IV and XIVa) is a fast and effective route by which VRE can achieve intestinal domination of the intestinal tract. The premise that anaerobic killing leads to VRE expansion is not a new one; a number of older studies using culture-based approaches demonstrate similar findings in other populations [41–43].

Interestingly, unlike metronidazole, administration of intravenous vancomycin is not clearly correlated with VRE colonization. In fact, intravenous vancomycin is less disruptive of the microbiota [21]; this may be related to the fact that gut penetration of intravenous vancomycin is often relatively low, especially if only given for several days [45, 46].

An ongoing subject for study is to determine if the microbiota can be protected against VRE domination, through colonization by specific robust commensal members. Some preclinical murine data indicate that colonization with *Barnesiella* spp., a group of Gram-negative anaerobes within phylum Bacteroidetes, is strongly resistant to VRE domination, and can be transplanted to a naïve gut to confer protection against domination [47].

51.3.2 Gram-Negative Bloodstream Infections

Systemic bloodstream infection with Gram-negative bacteria such as Enterobacteriaceae is of particular concern in patients with severe and/or prolonged neutropenia, which includes the pre-engraftment period in HCT. Prophylactic administration of fluoroquinolones can reduce the incidence of this infection, and is practiced at many transplant centers [48, 49]. Highly antibiotic resistant Gram-negative infections such as carbapenem-resistant Enterobacteriaceae (CRE) are of particular concern, and nosocomial transmission of these pathogens has been studied and documented, using detection of single-nucleotide polymorphisms using whole-genome sequencing [50]. However, transmission and/or colonization with Proteobacteria does not necessarily result in infection, and a robust microbial community can exert colonization resistance to resist expansion of these potential pathogens and maintain them at low abundances.

Study of the intestinal microbiota in HCT demonstrated that prophylactic fluoroquinolone administration markedly reduced the incidence of intestinal expansion by Gram-negative bacteria [21]. Furthermore, intestinal domination by aerobic Gram-negative bacteria is associated with subsequent development of Gram-negative bacteremia, providing confirmation of the gut origin of these infections.

The manner and circumstances in which Gram-negative bacteria achieve intestinal domination remains an active area of investigation. Intestinal inflammation and neutrophil recruitment into the gut may be contributing factors [51–53]. Commensal families such as Porphyromonadaceae, which resides within the Bacteroidetes phylum and contains *Barnesiella* spp. described in the prior section, offers some evidence that it may provide some degree of colonization resistance against pathogenic Gram-negative bacteria [54].

51.3.3 Viridans Group Streptococcal Bloodstream Infections

Viridans group streptococci are known to cause a particularly severe, life-threatening, toxic shock-like syndrome under certain settings of severe or prolonged neutropenia. Risk of this infection is very much determined by conditions and practices favoring oral mucositis, such as pre-engraftment phase of HCT and administration of high-dose cytosine arabinoside [55]. Since viridans group streptococci are known to be particularly abundant in the upper part of the gastrointestinal tract [28], systemic dissemination during neutropenia is most likely during administration of chemotherapy agents that favor upper gut mucosal damage [56]. Centers encountering this infection have adopted prophylaxis strategies to prevent these infections, such as administration of penicillin or vancomycin [57, 58]. Administration of intravenous vancomycin during the peri-transplant period in allo-HCT is currently a routine practice in some centers, and appears to be extremely effective [58], though current treatment guidelines for fever and neutropenia recommend against the routine use of Gram-positive agents such as vancomycin for prophylaxis [59].

It is notable that intravenous vancomycin provides effective prophylaxis against infection by viridans group streptococci, despite the fact that intestinal domination nonetheless occurs in approximately 40 % of HCT recipients, based on one study [21]. As discussed previously, intravenous vancomycin does not penetrate well into the gut lumen [45], so protection against viridans group streptococcal infections presumably takes place at the level of blood, in systemic circulation. This contrasts with fluoroquinolone prophylaxis against Gram-negative infections, where effects on the microbiota can be clearly seen.

51.3.4 *C. difficile* Infection

There is little doubt that *C. difficile* infection (CDI) results from disruption of the intestinal microbiota, due to antibiotic administration and other perturbations. In HCT recipients, rates of CDI are generally felt to be very high in comparison with other patient populations; reported CDI rates typically range from 12 % to 18 %, but can range greatly from as low as 5 % to as high as 30 % [60–66]. This may in part be a reflection of the degree of microbiota dysbiosis experienced by these patients.

Examination of the fecal samples of patients during HCT indicates that about 40 % of patients are asymptomatically colonized with toxigenic *C. difficile* at the start of HCT [66]. It is from this pool of pre-colonized recipients that CDI occurs, suggesting that the high rates of infection in this population cannot readily be explained by nosocomial transmission.

The exact factors and mechanisms that determine the risk of vegetative growth and toxin production by *C. difficile* in the transplant setting and other populations are still unclear. Patients in the non-transplant setting with recurrent CDI demonstrate relatively reduced microbial diversity in their feces [67]. Thus, as a means to restore this lost diversity, fecal microbiota transplantation (FMT) has gained notoriety as an effective way to cure patients with recurrent CDI, often succeeding in instances where other treatments have failed [26]. There are now several published forms of evidence for the effectiveness of FMT [27, 68, 69], including a randomized clinical trial demonstrating substantial benefits in the form of a durable cure [70].

Examination of the microbiota composition for evidence of protective elements against CDI has brought to light several commensal candidates that may have potential for treatment in the form of bacteriotherapy. *Bifidobacteria* spp. and *Lactobacillus* spp., both commonly formulated in commercial probiotic formulations, have shown some degree of evidence that they could offer protection from CDI [33, 71, 72]. However, these data are still considered limited, and CDI guidelines have not recommended the use of currently formulated probiotics [73].

Commensals from the Bacteroidetes phylum may have durable protective effects against CDI; in patients with recurrent CDI who were cured using FMT, examination of the microbiota before and after the procedure revealed that the most obvious microbial change was significant colonization with Bacteroidetes, where it had been previously completely lacking [26, 27]. Further evidence can be seen in fidaxomicin, which was shown to be non-inferior to oral vancomycin for the treatment of CDI, but with fewer observed recurrences [74]. This difference has been attributed to the fact that fidaxomicin specifically is capable of sparing *Bacteroides* spp. during treatment.

Members of family Lachnospiraceae (Clostridium cluster XIVa) have also demonstrated potential for protection against CDI. Colonization with one strain of Lachnospiraceae was able to confer colonization resistance against CDI in mice [18]. In a subsequent study, a mathematical model constructed from microbiota data from HCT recipients with and without CDI revealed highly distinctive protective effects from *C. scindens*, another member of Lachnospiraceae. Colonization of mice with *C. scindens* conferred protection against CDI [19]. The mechanism of protection was shown

to occur through production of secondary bile acids which were previously thought to inhibit vegetative growth of *C. difficile* [75, 76].

51.3.5 Graft-Versus-Host Disease (GVHD)

As early as the 1970s, the gut microbiota has been suspected to play a role in mediating outcomes in allogeneic HCT, particularly GVHD. Early studies in mice and humans suggested a link between the intestinal flora and propensity for graft-versus-host disease [77, 78]. This led to the practice of total or selective gut decontamination and environmental protection through laminar airflow isolation, which were done routinely at transplant centers in the hopes of preventing transplant-related complications. This was based on several initial studies which were promising [79, 80]. However, these practices failed to demonstrate consistent benefit over time in subsequent studies [81–83], and have largely fallen out of favor at most transplant centers.

More recently there has been renewed interest in findings means to prevent GVHD through modulation of the microbiota, given our better understanding of host immunity-microbiome dynamics. In studies of either mice or humans comparing the microbiome of individuals with GVHD with those without, a consistent pattern of microbial shift has been observed. Prospective studies have shown that individuals with GVHD have a preceding intestinal composition that is deficient in obligate anaerobes within Clostridia, regarded as healthy members that are beneficial for production of short chain fatty acids and prevent inflammation by upregulating regulatory T-cells through a variety of mechanisms [84, 85]. Instead, these individuals demonstrate domination by more oxygen tolerant microbes, including *Lactobacillus* spp., *Enterobacteriaceae*, and *Enterococcus* spp. [84, 86].

51.4 Solid Organ Transplantation (SOT)

Fewer studies of the microbiome have been performed in solid organ transplant compared with HCT. These patients are treated with fewer antibiotics and do not experience the initial fall in microbial diversity at the beginning of transplantation [87]. Nevertheless, several notable studies of solid organ transplant recipients have shown that the microbiome is clinically relevant and may have implications in medical management.

In renal transplant recipients, the intestinal microbiota was shown to influence the dosing of immunosuppressant medications. Specifically, patients colonized with *F. prausnitzii*, a commensal member of Clostridium cluster IV, required increased doses of tacrolimus in order to achieve therapeutic levels, compared with patients who were not colonized [87].

In another study of solid organ transplant recipients taking pharmacological immunosuppressants, evaluation of salivary microbiome revealed compositional differences among transplant recipients compared with control subjects not taking immunosuppressant medications. Specifically, patients taking corticosteroids and/or mycophenolate mofetil exhibited salivary samples with high microbial diversity [88]. This suggests that cell-mediated immune suppression may serve to create an oral environment that is more permissive of bacterial colonization with opportunistic pathogens, but without affecting other members through domination, in contrast to observations of HCT recipients.

In lung transplantation, a connection between the microbiota inhabiting airways and chronic lung allograft rejection has been hypothesized [89]. In this model, immune suppression must be carefully balanced; sufficient immune suppression is necessary to prevention allograft rejection, but allograft rejection is also strongly correlated with airway infection, which occurs if immune suppression is excessive. Furthermore, whether colonization of the airway with certain microbes confers higher or lower risk of lung allograft rejection has been a subject of interest. In one preliminary study, lung transplantation recipients showed higher bacterial diversity in lung fluid obtained by bronchoalveolar lavage, as compared with non-transplant controls [90]. Furthermore, compositional differences in lung microbiome were observed, including a strikingly higher abundance of Burkholderiaceae, a family within the Proteobacteria phylum, in lung transplantation recipients.

51.5 Summary and Future Directions

The initial observations thus far suggest that the microbiome is particularly important in the transplantation setting, having implications in immune status, risk of infection, and health of the allograft. Future microbiome studies will need to continue to examine and further define the full clinical implications of commensal microbes on transplant outcomes, and the underlying mechanisms behind these associations, in order to gain insights that can lead to potential interventions that can improve outcomes in transplantation.

Therapeutic interventions aimed at maintaining the microbiota in an ideal state during transplantation may be the logical result of these findings. Future consideration and exploration of these interventions are needed to bring the knowledge of the microbiome into practical importance.

For instance, an improved mechanistic understanding of the impact of antibiotics on commensal populations may help guide antibiotic stewardship more accurately. Based on observations thus far, emphasis on avoiding drugs with potent anti-anaerobic activity, and simultaneous de-emphasis of stewardship over drugs with minimal intestinal lumen penetration could be of benefit and should be further studied.

Replenishment of microbial populations following treatment-related perturbations with interventions such as fecal microbiota transplantation (FMT) may also be of benefit in certain transplant populations.

In addition to efficacy in the treatment of recurrent *C. difficile* infection, FMT could potentially serve to improve other transplant-related outcomes, such as transplant-related infections or GVHD. However, FMT has generally not been well studied in transplant recipients, and it is not yet clear whether additional safety concerns exist in treating these immune compromised patients in this manner. Given the uncertainty surrounding FMT, alternative therapeutic approaches to consider might include diet modification to induce conditions favorable for colonization with beneficial microbes (prebiotics), or bacteriotherapy either with single beneficial "keystone" members or with bacterial consortia. These approaches have been proposed as interventions that could be performed safely and effectively.

Additional areas of potential investigation includes study of the microbiota and risk for non-bacterial transplant infections (e.g., cytomegalovirus and adenovirus), relevance of commensal fungi and viruses (mycobiome and virome), and whether regional differences exist and are pertinent. Further study and understanding of these complex microbial populations and their interactions with human hosts can be used to inform transplantation practices and improve outcomes.

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52 Special Considerations for Long-Term Survivors After Hematopoietic Stem Cell Transplantation

Merav Bar and Mary E.D. Flowers

52.1 Introduction

Advances in hematopoietic cell transplantation (HCT) practices and supportive care have led to improved transplant outcome and an increasing number of long-term survivors [1]. Long-term (LT) transplant survivors are at risk for developing late complications as summarized in Table 52-1. Among 2 and 5 years HCT survivors, the main reported causes of death include relapse of the original disease, chronic graft-versus-host disease (GVHD), and late infections (Figure 52-1) [2–4]. This chapter focuses on late infections and infection-related complications that occur beyond day 100 after HCT. Late infections after transplant are generally localized to the skin, the upper respiratory tract, and the lungs [5]. Viral infections are responsible for more than 40 % of late infections after transplant, bacteria are responsible for approximately 35 % of infections, and fungi cause approximately 20 % of the late infections after transplant [6].

52.2 Risks for Late Infections After HCT

Late infections with bacteria, viruses, fungi, and other organisms are most common (1) in patients with delayed immune reconstitution, (2) in patients with chronic GVHD, (3) in recipients of cord blood transplant, (4) in recipients of T cell depleted allogeneic HCT, and (v) in CD34-selected autologous HCT recipients. The adoption of maintenance therapy with novel chemotherapy and immunomodulatory agents after autologous HCT for multiple myeloma [7–9] has also resulted in increased risk for late infection complications in this patient population as well [10–12].

It is standard practice to administer prophylaxis for infections caused by varicella zoster virus (VZV), *Pneumocystis jiroveci* (formerly *Pneumocystis carinii*), *Toxoplasma gondii*, and encapsulated bacteria (*Neisseria meningitidis, Haemophilus influenzae,* and *Streptococcus pneumoniae*) after HCT and to continue long-term prophylaxis beyond 100 days post-transplant in patients with chronic GVHD. However, no evidence based data is available to guide specific recommendations for duration of infection prophylaxis beyond 100 days after HCT, and practice is variable based on type of transplant, GVHD status, immunosuppression therapy, herpes serology status pre-transplant of patients and donors, diagnosis at transplant, prior treatment with certain chemotherapeutic agents, and prior history of infections.

A comprehensive review and guidelines for preventing infectious complications among HCT recipients, including beyond 100 days post-transplant, have been published and last updated in 2009 [13]. General practice for late infection prophylaxis at the Fred Hutchinson Cancer Research Center (FHCRC) and the Seattle Caner Care Alliance (SCCA) is summarized in Table 52-2. Vaccination is recommended in HCT long-term survivors to prevent infections (as discussed in detail in Chap. 48).

52.2.1 Immune Reconstitution After HCT

Reconstitution of the immune system after HCT is critical for both prevention and control of infectious disease and for prevention of recurrence of the original malignant disease for which the transplant was indicated.

Immune reconstitution following allogeneic HCT is a stepwise process. First, the innate immune system including epithelial barriers, monocytes, granulocytes, and natural killer (NK) cells recovers within weeks after transplantation. The adaptive immunity recovers significantly slower, and although B and T cell counts may normalize during the first months after transplantation, the B and T cell function may remain impaired for years [14].

The T cell compartment after transplant is repopulated through a thymus-dependent pathway by development of new lymphocytes from progenitor cells, and through thymus-independent pathway by peripheral expansion of residual mature donor and recipient lymphocytes by

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TABLE 52-1. Late (beyond day 100) complications after hematopoietic cell transplantation

Delayed immune reconstitution
Chronic graft-versus-host disease
Late infections
Autoimmune hematologic problems
Metabolic syndromes
Airway and pulmonary diseases
Cardiovascular complications
Gastrointestinal and hepatic complications
Musculoskeletal problems
Ocular problems
Dental problems
Growth and development impairment (children)
Endocrine problems
Central and peripheral nervous system problems
Recurrent malignancy
New cancers
Psychosocial effects

antigenic stimulation and homeostatic cytokines [15–17]. The thymus-independent pathway may recover within a number of months after transplant, while the thymus-dependent pathway may not fully function for years [18]. Thymus-dependent pathway depends on lymphoid-commitment progenitor cells derived from the hematopoietic stem cell graft as well as adequate thymus microenvironment for the development of T lymphocytes. However, the function of the thymus in a transplant patient is damaged in HCT recipients by age-related thymic involution, exposure to cytotoxic drugs or radiation, and GVHD [19], therefore the delay in the thymus-dependent immune recovery after transplant.

B cell count is low during the first couple of months after bone marrow grafting, but it subsequently increases and becomes supranormal by 1–2 years after transplant, as demonstrated in some studies [20]. Most B cells are naive and initially produce immunoglobulin M (IgM) rather than IgG or IgA [21].

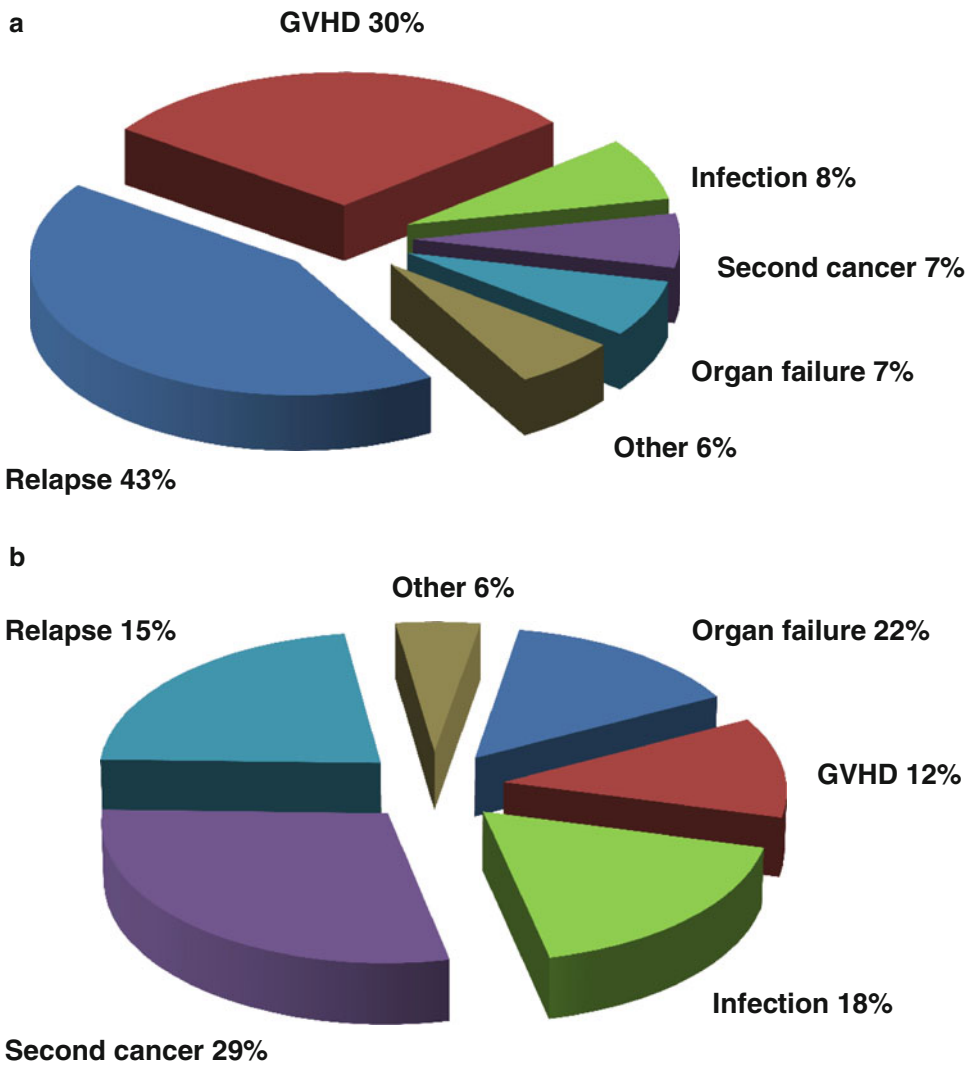

FIGURE 52-1. Causes of deaths after allogeneic HCT for hematological diseases. (**a**) COD among 2-year survivors. Represents abstracted data from two studies [2, 3]. (**b**) COD among 5-year survivors. Represents abstracted data from [4].

TABLE 52-2. Anti-microbial prophylaxis guidelines for adult patients after HCT at the Fred Hutchinson Cancer Research Center

Organism	Prophylactic agents	Prophylaxis after allogeneic HCT	Prophylaxis after autologous HCT
VZV	Acyclovir 800 mg twice daily OR Valacyclovir 500 mg twice daily	Continue for at least 1 year after transplant or 6 months after cessation of all immunosuppression, whichever occurs later.	One year after transplant. Longer prophylaxis if patient receives maintenance therapy post-transplant (e.g., bortezomib after transplant for multiple myeloma).
PJP	Trimethoprim/Sulfamethoxazole (TMP/SMX)—the most effective prophylaxis against PJP Double strength (DS) tablets of 160 mg trimethoprim and 800 mg sulfamethoxazole One DS tablet twice daily twice a week on 2 consecutive days It is recommended that patients with sulfa allergies will undergo desensitization. Alternative Prophylactic Therapy[a]: Dapsone 50 mg PO twice daily. Atovaquone 1500 mg oral suspension, once daily.	Continue for at least 6 months post-transplant in patients without chronic GVHD. Patients with chronic GVHD who are on immunosuppressive therapy may receive daily prophylaxis with TMP/SMX for both PJP and encapsulated bacteria prophylaxis for at least 6 months after completion of immunosuppressive therapy.	Continue for 6 months post-transplant.
Encapsulated bacteria	TMP/SMX—Double strength tablet once daily. Alternative prophylaxis[a]: Penicillin-based prophylaxis: Penicillin VK 500–750 mg twice daily. Other options: doxycycline, quinolones, and macrolides.	It is recommended that prophylaxis be started upon initiation of immunosuppression for chronic GVHD, and be continued until 6 months after discontinuation of all immunosuppression. Patients with active manifestations of chronic GVHD after discontinuation of all systemic immunosuppressive medications remain at increased risk for serious infections with encapsulated bacteria. Thus, continuation of prophylaxis against encapsulated bacteria as long as chronic GVHD manifestations remain active may be considered on a case-by-case basis.	
Toxoplasma	PJP prophylaxis with TMP–SMX has some activity against toxoplasmosis; For high-risk patient with TMP–SMX intolerant see text.	See for PJP section	See PJP section
Candida species	Fluconazole (400 mg/day)	Until day 75 post HCT or until discontinuation of steroids, whichever occurs later.	Until resolution of neutropenia and mucositis.
Mold (Aspergillus species, Zygomycetes)	Voriconazole Posaconazole may be considered for patients who receive high dose steroids for prolonged period of time since its greater activity against mold.	No data is available about the efficacy and toxicity of antifungal prophylaxis beyond day 100 post-transplant. Decision regarding late use of anti fungal prophylaxis should be made based on the patient's infection history, immunosuppression therapy, main organ function, etc. to assess potential benefit and risks for the individual patient.	

[a]As a general principle, alternative drugs should only be used if absolutely necessary, and should be prescribed for only short period of time. Potential reasons for alternative PCP prophylaxis include: severe neutropenia not explained by other causes, gastrointestinal intolerance, renal toxicity, and hepatotoxicity. Resumption of TMP–SMX prophylaxis should always be attempted as soon as possible. In the case of TMP–SMX allergy, desensitization should be attempted.

Many parameters have been shown to affect immune recovery after transplant as: (1) disease status at time of transplant; (2) patient/donor-specific factors (e.g., patient and donor ages, human leukocyte antigen (HLA) compatibility, and CMV serological status); (3) graft source and composition; (4) transplant-specific factors (e.g., use of granulocyte colony stimulating factor (G-CSF), immunosuppressive therapy, polyclonal antibodies as part of conditioning, presence and extent of GVHD, and CMV reactivation) [22–24].

52.2.2 Chronic GVHD

Chronic GVHD is a major cause of late morbidity and mortality after allogeneic HCT, mainly due to its inhibitory effect on immune recovery, and toxicities of glucocorticoids and other immunosuppressive agents used for GVHD treatment. Risk factors for the development of chronic GVHD are: use of a mobilized peripheral blood as the stem cell source, multiparous female donor into a male recipient, HLA mismatch, and old age [25]. Risks factors for chronic GVHD-related mortality are: direct progression from acute GVHD, platelet counts below 100,000/mcL at time of chronic GVHD diagnosis, hyperbilirubinemia, extensive skin disease, severe chronic GVHD by the National Institute of Cancer criteria, and low clinical performance score [26, 27]. If not treated adequately and in severe form of the disease, chronic GVHD can result in disability, major or recurrent infections due to delayed immune reconstitution, pulmonary insufficiency (due to bronchiolitis obliterans (BO) or restrictive lung disease related to scleroderma or fasciitis), skin ulcers, severe joint contractures, esophageal stenosis, vaginal stenosis, severe keratoconjunctivitis sicca, and others [28, 29].

Clinical and experimental data have shown that immune reconstitution is directly impaired by chronic GVHD due to destruction of bone marrow hematopoietic niches, inhibiting lymphopoiesis, and damage to the thymus microenvironment, which inhibits thymus-dependent T cell immunity [30–34]. Additional inhibition to immune recovery is mediated by the immunosuppressive therapy of chronic GVHD.

The mainstay of chronic GVHD treatment is glucocorticoids, but approximately 50% of patients require additional immunosuppressive agents for GVHD control [35, 36]. Most patients require immunosuppressive therapy for at least 2 years from the initial diagnosis of chronic GVHD, and approximately 10% of patients require continued immunosuppressive treatment beyond 5 years [37]. The prolonged use of glucocorticoids and other immunosuppressive therapy used after allogeneic transplant has significant effect on delayed immune reconstitution, including inhibitory effects on a broad range of specific immune responses mediated by T and B cells, as well as potent suppressive effects on the effector functions of phagocytes [38–40]. Because of their inhibitory effects on both acquired and innate immunologic function, the prolonged use of immunosuppressive agents significantly increases the risk of late infection after HCT [41–49].

52.2.3 Late Infection Risks After Cord Blood Transplant

Umbilical cord blood (UCB) transplantation is associated with delayed immune reconstitution, largely due to impaired thymopoiesis and late memory T cell function [50]. UCB contains mostly naive, antigen-inexperienced T lymphocytes, which do not transfer protective T cell memory function to the recipient. Additionally, UCB T cells display impaired capacity for effector cytokine production and reduced cytolytic activity [51, 52]. Consequently, UCB transplant is associated with delayed and incomplete immune reconstitution. As a result, infectious complications and viral reactivation represent the most prevalent causes of post-transplant morbidity and mortality in UCB transplant recipients [50, 53, 54].

52.2.4 Late Infection Risks After T Cell Depleted HCT

Depletion of T cells from the stem cell product (in vitro or in vivo) reduces the risk of GVHD by limiting the number of allo-reactive T cells but it also results in an increased risk of infections. In the first several months after HCT, the circulating T cell repertoire is essentially recruited from the progeny of the donor lymphocytes that are cotransplanted with the hematopoietic progenitor cells. Hence, in recipients of a T cell–depleted graft product, T cell–repertoire reconstitution is often delayed or incomplete compared with recipients of unmanipulated product. The limited size of the initial pool of T cells in combination with the length of time it takes the cells to repopulate in the recipient's blood is likely a major contributor for the lack of complexity in T cell repertoires in the first year after T cell–depleted HCT, and for the increased risk of late infections in this patient population [55]. Most common late infections after a T cell depleted HCT include those caused by herpesvirus, Epstein–Barr virus, and others [56–58].

52.2.5 Late Infection Risks After Haplo-Identical Transplant

Haplo-identical HCT offers the benefits of rapid and nearly universal donor availability, and has been accepted worldwide as an alternative for patients with hematologic diseases who do not have a completely HLA-matched sibling or who require urgent transplantation. Unfortunately, serious infections resulting from delayed immune reconstitution remain a major cause of mortality in haplo-identical HCT recipients, particularly in those receiving extensively T cell–depleted allografts [59, 60]. Haplo-identical HCT is characterized by prolonged B cell lymphopenia and low thymic function, which render patients susceptible to late viral and fungal infections [61]. The risk of late infections in recipients of un-manipulated graft from a haplo-identical donor with

post-transplant cyclophosphamide has not been compared to that of recipients of an un-manipulated graft from an HLA-matched unrelated donor using conventional immunosuppression post-transplant. However, a recent prospective cohort analysis of 70 recipients of un-manipulated haploidentical HCT with post-transplant cyclophosphamide demonstrates high incidence of viral infections/reactivations in the early and late post-transplant period, but with a quite low incidence of late bacterial and invasive fungal infections [62].

52.2.6 Late Infection Risks After Autologous HCT

Late infections are not common after an un-manipulated autologous HCT, but late herpesvirus infections are not uncommon in recipients of CD34+ selected grafts. Moreover, the increased use of novel drugs (i.e., proteasome inhibitor bortezomib and the immunomodulators thalidomide and lenalidomide) before and after autologous transplant has been associated with an increased risk for late viral infections in this setting, especially caused by herpesviruses [63–66].

52.3 Late Infections After HCT

52.3.1 Late Bacterial Infection

Encapsulated bacteria (*S. pneumoniae, H. influenza,* and *N. meningitidis*) are the most significant late bacterial infection after HCT. The most significant risk factor for late infections with encapsulated bacteria, particularly *S. pneumonia*, is chronic GVHD, due to persistent low levels of opsonizing antibodies, low CD4+ cell count, poor reticuloendothelial function, and long-term use of immunosuppressive therapy, especially corticosteroids, with their suppressive effects on phagocytosis [13, 67].

Optimal selection of prophylaxis should consider local patterns of pneumococcal resistance and individual allergies or toxicities associated with available antibiotic choices that include penicillin VK, trimethoprim–sulfamethoxazole (TMP–SMX; cotrimoxazole), second generation cephalosporins, quinolones, and azithromycin. None of these strategies has been tested in randomized trials but historical data indicate that fulminant septic events can occur without prophylaxis due to the profound immunosuppression [68]. Detailed recommendations for prevention of late bacterial infections (i.e., beyond 100 days after transplant) can be found in the 2009 international guidelines for prevention of infection after HCT [13].

Due to the emergence of penicillin resistance encapsulated organisms, and the concomitant need for *Pneumocystis jiroveci* pneumonia (PJP) prophylaxis in patients with chronic GVHD some experts favor TMP–SMX given as one double strength tablet once daily as the first-line drug for both chemoprophylaxis for infections with encapsulated bacteria and PJP for patients with chronic GVHD. If TMP–SMX is not tolerated, second generation cephalosporins, quinolones, and azithromycin prophylaxis may be substituted for coverage of encapsulated organisms, and dapsone, atovaquone, or aerosolized pentamidine added as alternative for PJP prophylaxis [13]. Encapsulated organism prophylaxis after transplant may also be considered in patients without GVHD but receiving glucocorticoid, patients with persistent or recurrent manifestations of chronic GVHD without ongoing use of immunosuppressive medications, patients who are receiving treatment for malignancy after transplant, and asplenic patients. At our center, chemoprophylaxis for encapsulated organisms is continued for at least 6 months after discontinuation of all immunosuppression medications for patients with a chronic GVHD history. For patients with prior splenectomy, the chemoprophylaxis is continued until age 6 OR 2 years after splenectomy, whichever occurs later. While continuation of chemoprophylaxis until 6 months after discontinuation of systemic immunosuppression represents the opinion of some experts and is not evidence based, such recommendation is based on the observation that most exacerbation of chronic GVHD occurs within 6 months after discontinuation of primary immunosuppressive treatment [35, 36], and the speculation that flare of GVHD may be triggered by infections via upregulation of alloreactivity associated with its proinflammatory milieu.

Late bacterial infections after transplantation may also be due to other organisms, such as Gram positive and Gram negative aerobic bacteria [69–71]. Therefore, empirical antibiotic treatment in HCT survivors with clinical suspicion for infection should include broad-spectrum coverage until the infecting organism is identified.

Severe hypogammaglobulinemia (i.e., IgG levels <400 mg/dL) can be associated with bacteremia or recurrent sinopulmonary infections. While routine monthly intravenous immunoglobulin (IVIG) administration to HCT recipients beyond 100 days after allogeneic or autologous HCT is not generally recommended as a means of preventing bacterial infections, administration of IVlG to maintain IgG levels higher than 400 mg/dL may be considered for patients at risk of late bacterial infections (e.g., patients with chronic GVHD and those with severe hypogammaglobulinemia) [13, 68]. At our center, IVlG is recommended during the first year after allogeneic HCT for patients with IgG levels of 400 mg/dL or less if other risk factors are present. IVlG is also recommended for patients with chronic GVHD with recurrent sinus/pulmonary infections with low serum IgG levels. Some experts recommend monitoring IgG levels and administering IVlG in patients with chronic GVHD and IgG levels lower than 400 mg/dL regardless of prior history of recurrent sinus/pulmonary infections or type of systemic immunosuppression treatment used, but there are no data demonstrating that this approach improves outcome [72].

52.3.2 Late *Pneumocystis jiroveci* Pneumonia (PJP)

P. jiroveci pneumonia (PJP), formerly known as *Pneumocystis carinii* pneumonia (PCP), used to be a common opportunistic infection in immunocompromised patients. However since effective PJP prophylaxis is routinely used after HCT, PJP infection after HCT is infrequent. The rare cases of late PJP after transplant are usually due to non-compliance or inadequate prophylaxis. The 2009 international guidelines for infection prevention after HCT recommend PCP prophylaxis from engraftment until at least 6 months after allogeneic HCT, with longer prophylaxis in patients who continue to receive immunosuppressive drugs [13]. At our center, PJP prophylaxis is given at least for 6 months after autologous transplant, and for 6 months after discontinuation of all immunosuppression after allogeneic transplant. TMP–SMX is the most effective PJP prophylaxis, thus generally accepted as the first choice for this purpose [73, 74]. Approximately 15–30 % of patients require therapy other than TMP–SMX because of drug allergy, gastrointestinal intolerance, renal or liver toxicity, and possibly marrow suppression. While breakthrough cases of PJP can occur with all alternative agents, use of daily dapsone is associated with acceptable prevention [75]; limited data exist on atovaquone in the allogeneic HCT setting [13] and breakthrough cases have been observed [75]. Pentamidine can be given as aerosol or intravenously, but it appears to be less effective [76, 77].

52.3.3 Late Toxoplasma Infection

Toxoplasmosis, caused by *Toxoplasma gondii*, is a potential fatal opportunistic infection following allogeneic HCT [78]. Toxoplasmosis after HCT is rare, and nearly exclusively develops as reactivation in seropositive transplant recipients who did not receive adequate prophylaxis [78, 79]. The incidence of *Toxoplasma gondii* disease varies according to the seroprevalence in the geographic area. Toxoplasmosis usually presents as encephalitis 2–6 months after transplantation in the setting of severe T-lymphocyte deficiency, but later occurrence has also been reported in patients receiving long-term immunosuppression [78, 80, 81]. Dissemination with encephalitis, myocarditis, pneumonitis, hepatitis, and other sites may occur and worsens the prognosis. The preferred regimen for Toxoplasma prophylaxis is TMP–SMX, which is routinely used for PJP prophylaxis after transplant. The efficacy of other PJP prophylactic drugs (e.g., dapsone and atovaquone) for *Toxoplasma* prevention is unknown [82–85]. The international guidelines for infection prevention after HCT recommend a strategy for prevention of *Toxoplasma gondii* in high-risk patients who are intolerant to TMP–SMX [13]. According to these guidelines, such patients can either receive prophylaxis with one or more drugs effective against *Toxoplasma gondii* (i.e., clindamycin, pyrimethamine plus leucovorin, pyrimethamine plus sulfadiazine, or pyrimethamine and sulfadoxine plus leucovorin) or be monitored with PCR testing and given preemptive treatment [86]. However, there are no published prospective studies on the benefit/risk ratio of such strategies.

52.3.4 Late Viral Infections

52.3.4.1 Varicella Zoster Virus Infection

Varicella zoster virus (VZV) disease is a common late viral infection after HCT [87]. VZV can present with skin or visceral disease. Although most VZV disease after HCT is caused by reactivation of endogenous VZV, HCT recipients who are VZV seronegative, or VZV seropositive and immunocompromised, should be advised to avoid exposure to persons with active VZV infections. In a randomized, double-blind trial, oral acyclovir at doses of 800 mg twice daily for 1 year after transplant prevented VZV infection after allogeneic HCT without rebound disease after discontinuation of prophylaxis [88]. The international guidelines for infection prevention after HCT recommend acyclovir to prevent VZV infection for the first year after HCT in VZV-seropositive allogeneic and autologous HCT recipients [13]. Acyclovir prophylaxis may be continued beyond 1 year in allogeneic HCT recipients who have chronic GVHD or require systemic immunosuppression. The optimal duration of prophylaxis is poorly defined in patients with chronic GVHD, as there appears to be a persistent risk of VZV reactivation disease even after discontinuation of all systemic immunosuppressive treatments and with CD4 cell count exceeding 200 cells/mL [13, 89]. At our center, acyclovir is given prophylactically to all allogeneic transplant recipients for 1 year after HCT or at least 6 months after discontinuation of all systemic immunosuppressive medications, whichever occurs later, and for 1 year after autologous transplant. According to the international guidelines for infection prevention after HCT the recommended dose of acyclovir prophylactic is 800 mg twice a day [13]; however, lower doses may be sufficient for VZV prophylaxis [90–92].

52.3.4.2 Cytomegalovirus Infection

Cytomegalovirus (CMV) disease usually occurs during the first year after HCT, but may occur later in patients with ongoing immunosuppression [93, 94]. Gastroenteritis and pneumonia are the most common late manifestations of CMV disease, but few cases of retinitis, encephalitis, and marrow failure have been reported [94–97]. CMV seropositive recipients of allogeneic transplants are at the highest risk of developing CMV infection and disease [98, 99]. Other groups at increased risk for the development of CMV disease are autologous HCT recipients of a CD34-selected graft.

Due to potential toxicities, no CMV prophylaxis is usually given to patients after HCT except for UCB transplant recipients, which represent the highest risk group for late CMV reactivation and disease [100]. Routine CMV blood monitoring and preemptive therapy, however, are recommended and useful for all patients at risk for late CMV disease. Polymerase chain reaction (PCR) testing is the standard assay in the USA for CMV surveillance in blood in high-risk patients. The assay is highly sensitive and provides reliable quantitative measurements of CMV load in plasma. While antigenemia testing also performs well for CMV monitoring, quantitation and sensitivity may be affected by delay in performing the test from specimen shipped from a distant location.

Studies have demonstrated that certain allogeneic transplant recipients are at increased risk for late CMV disease and CMV-related mortality after HCT; these patients include those treated for CMV infection in the first 100 days after transplantation, CMV seropositive recipients of cord blood transplants, and those treated with corticosteroids or other systemic immunosuppressive agents for chronic GVHD [101–103]. Continued monitoring for CMV reactivation after 100 days following HCT is recommended for these high-risk patients on a weekly basis. Weekly testing is recommended due to the rapid replication kinetics of virus in these immunocompromised hosts, and because CMV disease can develop in the interval between a negative assay and the next sample if the tests are spaced 2 weeks apart. Few studies examined when late surveillance can be safely discontinued. In our practice, non-cord umbilical blood HCT recipients who are not receiving high levels of immunosuppression (e.g., < 1 mg/kg/day corticosteroids or treatment with mycophenolate mofetil) and who have had three consecutive negative surveillance tests after day 100 post-transplant can be changed to every other week blood CMV monitoring; if taper of immunosuppression continues and patient remains negative, CMV blood testing may be stopped after two additional tests. If treatment with corticosteroids is increased or secondary therapy for chronic GVHD is added, particularly initiation of treatment with mycophenolate mofetil, we resume CMV monitoring on a weekly schedule.

CMV seropositive UCB transplant recipients may be at increased risk for developing CMV reactivation and disease. Despite using standard preemptive strategies in CMV seropositive UCB transplant recipients, an evaluation of this population at our center revealed that almost 100 % reactivated CMV and 25 % developed CMV disease [100]. While some centers have reported lower rates of CMV reactivation and disease, comparable with standard allogeneic transplants [104], others also have found high rates of CMV reactivation in the UCB population [105]. Therefore, we recommend prophylactic CMV therapy in addition to virologic surveillance and preemptive therapy in UCB transplant recipients at risk. The preferred CMV prophylaxis beyond day 100 after HCT for cord blood recipients with a prior history of CMV reactivation in the early post-transplant phase is valganciclovir 900 mg PO once daily [106]. This regimen, while effective, may not be tolerated due to the risk of neutropenia. In these cases, we have used a modified regimen of high dose valacyclovir (oral valacyclovir 2 g three times daily) [107]. This regimen is also our preferred CMV prophylaxis for cord blood recipients who did not experienced early CMV reactivation (i.e., before 100 days after transplant). For patients who are unable to take oral formulations and who are deemed at high risk for late CMV disease, intravenous regimens of acyclovir (500 mg/m2 IV every 8 h) [107] or ganciclovir 5 mg/kg/day are used [108].

Several studies indicate that preemptive treatment is effective to prevent CMV disease and improve outcome after HCT [109, 110]. In a randomized trial, preemptive CMV therapy in patients with plasma CMV DNA levels of 1000 IU/mL or rising DNA levels (i.e., greater than 5 times of baseline level within 1 month) was as effective as prophylaxis for prevention of late CMV disease [106].

Since the risk of CMV acquisition via blood products continues late after transplantation, all blood products given to seronegative HCT recipients should be CMV negative or leukocyte reduced [13].

52.3.4.3 Respiratory Virus Infections

Patients with and without chronic GVHD are at risk for acquisition of late respiratory tract infections such as those caused by influenza virus, parainfluenza virus, human metapneumovirus, and respiratory syncytial viruses; however, lower respiratory tract complications appear to be higher in patients with chronic GVHD. Respiratory virus infections represent a major contributor to development of severe pulmonary airflow decline and are associated with risk of developing lung GVHD (i.e., bronchiolitis obliterans) [111–117]. Inactivated Influenza vaccine is recommended after HCT as early as 6 months after HCT and for individuals with close contact with the transplant recipient (as discussed in detail in Chap. 48) [118].

52.3.4.4 Other Virus-Related Late Complications

Other potential viral-related late complications after HCT are chronic hepatitis and cirrhosis due to hepatitis B and C infection (as discussed in Chap. 37), BK virus associated late hemorrhagic cystitis, human papilloma virus infection, EBV lymphoproliferative disease (discussed in Chap. 26), and recurrent HSV disease, especially in patients receiving no or insufficiently doses of acyclovir prophylaxis (discussed in Chap. 29). No routine surveillance of such viral infections is recommended, but specific evaluation should be performed if clinical suspicion is raised, and patients should be treated accordingly.

52.3.5 Late Fungal Infections

Late fungal infections are most common in allogeneic HCT recipients receiving high doses of corticosteroids. Antifungal prophylaxis beyond 100 days following allogeneic HCT may be considered, especially in patients with chronic GVHD who are receiving high dose corticosteroids (i.e., greater than 1 mg/kg/day prednisone or equivalent) or other immunosuppressive agents. While little evidence-base recommendations are available for antifungal prophylaxis beyond 100 days after transplantation, routine use of antifungal prophylaxis in the early period after allogeneic HCT has been shown to decrease invasive fungal infections from 12 % to 4 % [119]. Invasive aspergillosis remains, however, a principal cause of infection-related mortality after HCT, with the majority of cases occurring beyond day 100 after transplant [120–122], and with main risk factors being chronic GVHD and prolonged treatment with high dose corticosteroids [120, 121]. While evidence-base is lacking for prolonged use of voriconazole or posaconzole prophylaxis beyond day 100 after transplant, the National Institute Cancer chronic GVHD Supportive Care consensus recommends antifungal prophylaxis in patients with chronic GVHD receiving high dose corticosteroids [68]. Until randomized studies to evaluate the safety of prolonged voriconazole or posaconzole prophylaxis are conducted, the decision whether to start or continue prophylaxis with either of these agents in patients who receive high dose immunosuppression beyond 100 days after transplant should be determined by the treating physician based on the patient's clinical status, expected duration of high dose corticosteroids therapy, and prior history of fungal infections.

52.4 Long-Term Follow-Up Recommendations After HCT

Long-term clinical monitoring is necessary to identify early signs of chronic GVHD, infections, and other potential late complications after transplant, in order to prevent severe morbidity and mortality. Table 52-3 summarizes the FHCRC guidelines for long-term follow-up post-transplant.

Routine monitoring and supportive care directed at organ-specific complications are crucial to the management of late complications after HCT. While most of long-term monitoring and supportive care guidelines for patients beyond 100 days after HCT are based on expert opinion rather than controlled studies, many of our practices at the FHCRC have been evaluated retrospectively [88]. The FHCRC long-term post-transplant general guidelines are systematically updated based on the published literature and can been found at the following link: http://www.fredhutch.org/en/treatment/long-term-follow-up/information-for-physicians.html.

TABLE 52-3 General long-term follow-up recommendations for adult patients after HCT at the Fred Hutchinson Cancer Research Center

1. Screening for secondary malignancies. Routine oncologic evaluation at yearly intervals is recommended due to increased risk of new malignancies after HCT. Annual oncologic screening as per recommendations to the general population and additional screening including yearly oral exam by a dentist and an yearly skin thorough exam due to the increased risk for head, neck, and skin cancer after HCT. Secondary malignancies rarely occur earlier than 2 years after transplantation, but the risk of developing cancer increases progressively after 5 years.
2. In patients transplanted for chronic myeloid leukemia or Philadelphia chromosome-positive acute lymphoblastic leukemia, routine monitoring for *BCR/ABL* transcripts by molecular testing of a blood sample is recommended every 6 months for the first 2 years after transplant, and yearly thereafter if the results remain negative. If *BCR/ABL* is positive in blood, examination of bone marrow by conventional morphology, cytogenetic analysis, molecular studies for the *BCR/ABL*, and chimerism is recommended.
3. Measures to prevent osteoporosis in women and all patients receiving long-term treatment with corticosteroids include:
   - Calcium 1500 mg/day in the diet or in supplements to meet daily requirements
   - Vitamin D 800 IU/day in the diet or in supplements to meet daily requirements
   - Daily weight-bearing exercise for 20–60 min
   - Dual photon densitometry at day 80–100 post-transplant evaluation and yearly thereafter
   - Sex hormone replacement therapy if levels are low and the benefits are thought to offset the risks.
4. Pulmonary function tests (PFTs) at day 100 post-transplant, at time of initial diagnosis of chronic GVHD, 1 month after resolution of respiratory virus infection, at 1 year after allogeneic HCT, and after 1 or 3 months if there has been abnormal testing in a previous time period or if there are new signs or symptoms concerning for pulmonary disease. PFTs should be monitored every 3–6 months during the first 2 years after the initial diagnosis of chronic GVHD or if clinically indicated.
5. Thyroid function testing annually, especially in patients who have received prior irradiation.
6. Ophthalmologic examination at annual intervals.
7. Immunizations (see Chap. 48).

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53 Special Considerations for Long-Term Survivors After Solid Organ Transplantation

Hakim Azfar Ali, Scott M. Palmer, and Oriol Manuel

53.1 Introduction

The field of solid organ transplantation has made remarkable progress in the second half of twentieth century starting from experiments in skin transplants to successful transplantation of visceral organs in common practice. It has come out of the shadows, to change from an experimental treatment modality to a mainstream therapeutic option, for treatment of multiple end stage disease processes.

This change in scene for organ transplantation has been driven by multiple factors including better immunosuppressive medications, better surgical technique, a more in depth knowledge of immunology and advanced support modalities to get patients through surgery in addition to the development of better antibiotics and preventive strategies to reduce the burden of infectious complications. The introduction of 6-mercaptopurine in 1950s and azathioprine in 1960 provided a boost to immunosuppression and the introduction of calcineurin inhibitor cyclosporine in 1980s markedly improved graft survival. Meanwhile the surgical techniques were developed and improved upon to decrease the early post-operative complications.

Early post-operative morbidity and mortality in solid organ transplant (SOT) recipients still remains a daunting challenge. Besides the direct surgical complications this also stems from other factors including early graft dysfunction, acute and hyperacute rejection, ischemic injury, early infections, and drug toxicity. However as these immediate and early post-operative issues are improving, more organ transplant recipients are surviving beyond one year.

The median survival of SOT recipients has improved remarkably to 12.1 years for a kidney transplant, 10.1 years for a liver transplant, and 5.1 years for lung transplant. Liver transplant outcomes at 1 year have improved from a dismal 30% in the pre-cyclosporine era to more than double in the post-era [1] and are now at over 90%. Similar results were observed in kidney and cardiac transplant [2] fields after introduction of cyclosporine and tacrolimus. Long-term graft failure at 10 years post-transplant has declined to an all-time low (Figures 53-1 and 53-2) (http://m.srtr.org/2011/chapters/lung/adults/outcomes.aspx).

As SOT recipients survive longer the focus is shifting to include delayed causes of morbidity and we are recognizing a large number of long-term complications which in some cases can be organ and life threatening. The definition of long-term complications is arbitrary and can be taken to mean complications occurring after 3 months or after a year from surgery. In any event, these complications have serious implications for the quality of life of organ transplant recipient.

The common complications range from long-term side effects of the immunosuppressant medications to development of malignancies, effects of chronic rejection, and late-onset infections. Other complications that often get overlooked include metabolic and cardiovascular diseases similar to the non-transplant patient except that some of these have an altered course and progression in the organ transplant recipients (Table 53-1). Some of these complications are common across the board for all solid organs and others are more specific to the organ transplanted (Table 53-2).

53.2 Late-Onset Infections After Solid Organ Transplantation

Most of the infectious complications developing after transplantation occur during the first weeks post-transplant, and it is within this period when most preventive strategies are set up to decrease the burden of infection [3]. Because of the prolonged survival of SOT recipients observed over the last decades, some infections may now appear very late after transplant. While these infections are usually community acquired, some patients may still present opportunistic infections with atypical presentations. Unfortunately, there

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is no extensive literature about the epidemiology and clinical characteristics of infection occurring in stable SOT recipients, months or years after transplantation. Follow-up of patients included in randomized controlled trials usually stops at 12 months post-transplant. In prospective cohorts, the capture of infectious episodes is usually more challenging than early after transplant, when the patient is followed by the original transplant center. In a large Spanish cohort, the incidence of late infection (>6 months) was eight times lower than during the first 6 months post-transplant (from 0.4 to 3.5 episodes of infection per 1000 transplantation days, respectively) [4]. In this study, risk factors for late infection were lung transplantation, previous bacterial infection, surgical complications, acute rejection, and chronic allograft dysfunction, highlighting a subgroup of patients at higher risk for health-care associated and opportunistic infections.

Bacterial infections are the most common infectious complications after transplantation, and represent approximately 70–80% of all infections. *E. coli*, *Enterococci*, and *Pseudomonas* are the most prevalent pathogens involved in these infections. Late-onset bacterial urinary tract infections (UTI) are particularly frequent in kidney transplant recipients [5]. The impact of these late infections in impairing allograft outcomes has not yet been elucidated; while in some studies, late recurrent UTI seemed to be associated with an increased risk for allograft dysfunction and loss [6, 7], this has not been found in other studies [8]. Also, bacterial pneumonia is a significant cause of community-acquired infection after lung transplantation [9]. Incidence of pneumococcal disease is estimated to be more than 10 times

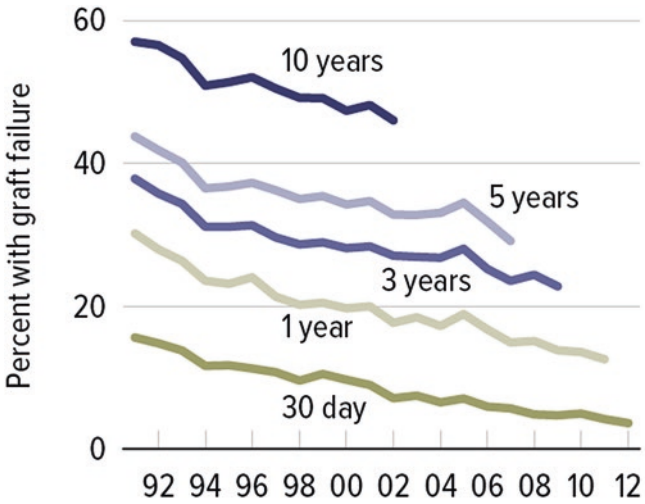

FIGURE 53-1. Graft failure amongst adult liver transplant recipients: deceased donor. Annual Data Report 2012, Scientific Registry of Transplant recipients (Liver), Figure LI 6.2 (http://srtr.transplant.hrsa.gov/annual_reports/2012/Default.aspx). Accessed 10/29/15.

TABLE 53-1. Late-onset complications after solid organ transplantation

- Infectious
- Non-infectious
  - Chronic kidney disease
  - Neoplasms
  - Metabolic complications
    - Hypertension
    - Hyperlipidemia
    - Diabetes
    - Obesity
  - Bone disease
  - Hematologic complications
  - Neurologic and neuropsychiatric
  - Misc. drug effects, e.g., tremor
  - Chronic allograft dysfunction
  - Native disease recurrence

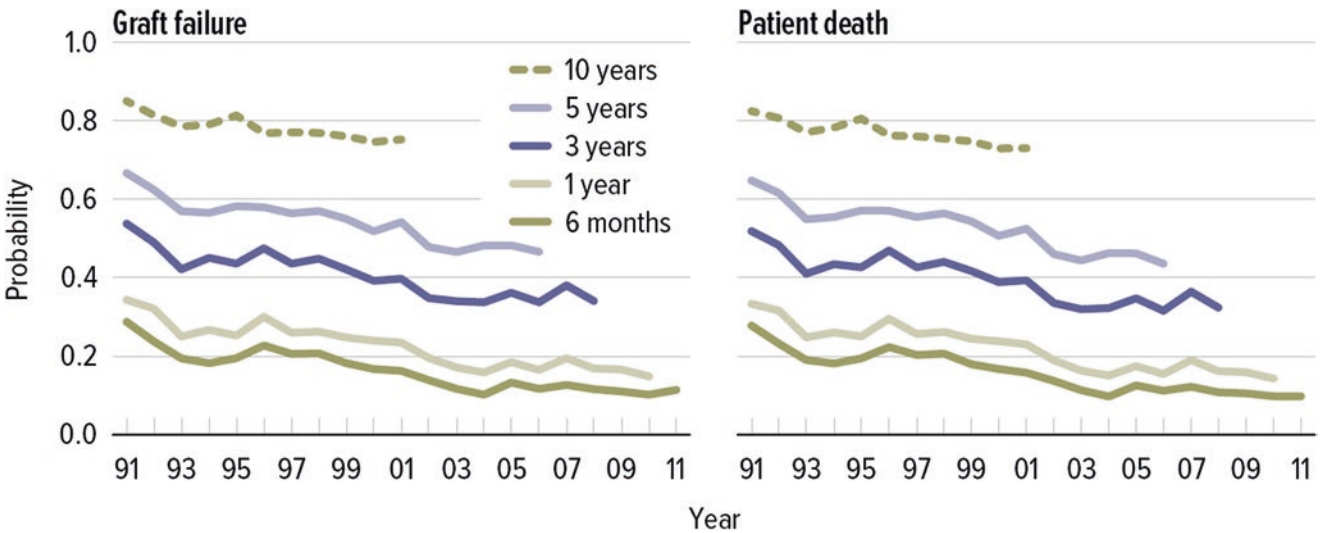

FIGURE 53-2. Graft failure and patient death amongst adult lung transplant recipients. Lung Adult Transplant—Outcomes, Scientific Registry of Transplant Recipients, Figure 5-2 (http://m.srtr.org/2011/chapters/lung/adults/outcomes.aspx). Accessed 7/25/15.

higher in SOT recipients than in the general population, and it is a cause of late infections [10]. In a large Canadian cohort, median time to presentation of pneumococcal disease was 2.75 years post-transplant, with a range reaching up to 24 years post-transplant. The efficacy of novel vaccination strategies using the 13-valent conjugated pneumococcal vaccine should be evaluated in SOT recipients [11]. Tuberculosis may reactivate (or being acquired) late after transplant [12]. Transmission of tuberculosis may occur through the liver, lung, or kidney grafts as well besides reactivation of the recipient disease particularly in cases of bilateral lung transplantation [13]. Because tuberculosis may present with atypical manifestations and because treatment is challenging due to drug–drug interaction and toxicity, it is associated with significant morbidity in SOT recipients. Diagnosis and therapy of latent tuberculosis infection in transplant candidates and in contact transplant recipients are of paramount importance to decrease the burden of the disease after transplant [14]. Listeria in SOT recipients is rare (0.12% in a Spanish series), presenting late after transplant (>50% of cases presenting after 6 months post-transplant) and associated with significant mortality [15]. Risk factors associated with listeriosis include diabetes, use of high-dose steroids, and absence of cotrimoxazole prophylaxis.

Viral infections are a common complication after transplantation. Cytomegalovirus (CMV) remains associated with significant morbidity in SOT recipients and the incidence of CMV replication may reach up to 70–80% in high risk patients (seronegative recipients receiving an organ from a seropositive donor), and approximately 15–30% of those patients will develop CMV disease [16]. CMV disease currently develops in the weeks following the discontinuation of antiviral prophylaxis, usually during the first year post-transplant. However, a minority of patient may develop CMV disease even later on after transplant. In a randomized controlled trial comparing 3 vs. 6 months of antiviral prophylaxis, 12% of all episodes of CMV disease were diagnosed between 1 and 2 years post-transplant [17]. The causes of this late onset of CMV infection are not completely understood, but it can be related with enhanced immunosuppression due to chronic rejection, delayed cell-immune control in patients receiving long-term antiviral prophylaxis, immunosenescence in older recipients, or late primary infection acquired by natural transmission, such as in case of contact with virus shed from the upper respiratory tract from children or by sexual contact. Interestingly, patients with very late-onset CMV disease usually have atypical manifestations, such as chronic gastrointestinal involvement with low viral loads in blood [18].

TABLE 53-2. Probability of long-term survival of adult lung transplant recipients

		One-year events, 2008–10 tx		Five year events, 2004–06 tx	
	Level	*N*	%	*N*	%
Bronchiolitis Obliterans syndrome (BOS)	Grade 3	36	0.8	356	9.7
	Grade 2	32	0.7	170	4.6
	Grade 1	85	1.9	279	7.6
	Grade OP	107	2.3	263	7.2
	Grade unk.	110	2.4	485	13.2
	No	3746	82.2	1972	53.9
	Unk.	441	9.7	136	3.7
Renal dysfunction	Yes	807	17.7	1737	47.4
	No	3492	76.6	1836	50.2
	Unk.	258	5.7	88	2.4
Hypertension, drug-treated	Yes	1570	34.5	2319	63.3
	No	1660	36.4	874	23.9
	Unk.	1327	29.1	468	12.8
Diabetes	Yes	878	19.3	1654	45.2
	No	3412	74.9	1914	52.3
	Unk.	267	5.9	93	2.5
Malignancy	Yes	159	3.5	563	15.4
	No	4131	90.7	3039	83.0
	Unk.	267	5.9	59	1.6
Re-hosp.	Yes	2402	52.7	3064	83.7
	No	1814	39.8	483	13.2
	Unk.	341	7.5	114	3.1
Total		4557	100.0	3661	100.0

Lung Transplant—Outcomes, Scientific Registry of Transplant Recipients, Figure 5-7 Post-transplant events among adult lung transplant recipients (http://m.srtr.org/2011/chapters/lung/adults/outcomes.aspx). Accessed 7/25/15.

Some cases of very late CMV replication due to drug-resistant viruses after exposure to antiviral agents have been described [18]. There are no proposed screening strategies for CMV after the second year post-transplant, so that a high index of suspicion is needed in case of unexplained clinical symptoms compatible with CMV disease.

Incidence of zoster is increased up to 3–5 years post-transplant and can be associated with significant morbidity, such as postherpetic neuralgia [19]. BK virus associated nephropathy is a common cause of chronic allograft dysfunction in kidney and kidney–pancreas transplant recipients. BK virus associated nephropathy generally appears during the first 12–24 months after transplantation, but series of late-onset BK virus nephropathy has been described, particularly in patients with enhanced immunosuppression [20, 21]. Infection by community-acquired respiratory viruses, such as influenza virus, RSV, PIV, or rhinovirus, may occur in any post-transplant period [22]. Although time from transplant appears to have a major impact on the outcome of influenza in SOT recipients (with high mortality in the early post-transplant period), cases of allograft dysfunction following influenza have also been observed late after transplant [23]. Thus annual vaccination with influenza vaccine remains highly recommended in all SOT recipients [24]. HPV infections are a significant problem after transplantation, due to an increasing incidence of oropharyngeal and genital cancer. Vaccination against HPV of young male and female transplant candidates and recipients is thus of importance to decrease the burden of HPV related cancers [25]. Other late viral infections reported in the literature include HHV-8 and EBV-related proliferative disorders. Chronic hepatitis E virus (HEV) infection has recently described as a problem in SOT recipients in endemic regions, probably related with consumption of undercooked game/pork meat, although the epidemiology of this infection in the transplant setting is not completely known [26].

Fungal infections can manifest late after transplant. Because of the direct contact of the lung allograft with the environment, lung transplant recipients are particularly at increased risk of developing invasive fungal infection. Risk factors for late aspergillosis have been described, and include chronic allograft dysfunction, previous respiratory infection, and need for renal replacement therapy [27]. Invasive candidiasis is particularly frequent during the post-operative period and much less frequent afterwards; no studies have assessed the prevalence of candidiasis late after transplantation. There are also scarce data on the epidemiology of other invasive fungal infections, such as mucormycosis, cryptococcosis, and black molds. Cases reported usually occur in oversuppressed patients due to chronic rejection.

Because of the lower incidence of infection in SOT recipients and the absence of well-defined risk factors, preventive strategies of infection in long-term survivors after transplantation are not well established. An important point is to identify patients with incomplete vaccination schedule and to administer missing vaccines after transplantation [11]. Specific teams dedicated to assess the vaccination status of transplant candidates and recipients can improve the administration of these vaccines. Because cotrimoxazole is usually well tolerated and cheap, long-term prophylaxis with this drug is widely used. In particular, patients with sustained low lymphocyte counts may benefit from extended duration of prophylaxis. Some authors suggest screening for hypogammaglobulinemia, hypocomplementemia, and low lymphocyte subpopulation counts to identify patients at higher risk for infections. Antibiotic prophylaxis (besides cotrimoxazole) for prevention of recurrent urinary tract infections (UTI) should be evaluated in an individual basis in kidney transplant recipients. The reduction on the number of episodes of UTI should be balanced with the acquisition of antibiotic resistance, particularly with the use of quinolones [28]. The experience with other drugs for UTI prophylaxis in kidney transplant recipients, such as fosfomycin or nitrofurantoin, is lacking [29]. Long-term antiviral or antifungal prophylaxis is not generally recommended, except in selected patients with recurrent infections (for example, acyclovir for HSV infection), or at high risk for a particular infection (for example, lung transplant recipients with environmental risk for aspergillus infection). In some settings, such as in lung transplantation, long-term inhaled antifungal or antibiotics may be preferred to avoid safety concerns associated with the administration of systemic antibiotics [30].

53.3 Renal Failure/Chronic Kidney Disease

Chronic kidney disease (CKD) is a common complication of renal and non-renal solid organ transplants and serves as a significant cause of morbidity and mortality, besides driving up the healthcare costs of solid organ transplantation. There is a lot of variability in the reported incidence of chronic renal failure in this population because of the lack of defining parameters and inconsistencies in reporting and definition. The incidence of renal dysfunction reported in non-renal organ transplant recipients varies from 10% to 80% [31]. As the longevity of the solid organ recipients increases a large majority of them will develop some degree of chronic renal disease.

An analysis of 70,000 non-renal transplant recipients from the US SRTR registry found an adjusted risk of 20–25% of Stage IV to V CKD at 5 years [32]. In this study cumulative incidence of severe renal failure (defined as creatinine clearance less than 29 ml/min/1.73 sq m BSA) at 5 years was found to be 18.1% in liver, 10.9% in heart, 15.8% in lung, 6.9% in heart–lung, and 21.3% in intestinal transplant recipients. The majority of renal transplant recipients have a higher creatinine and a lower GFR at 1 year.

CKD complicates the care of SOT recipients both directly by increasing hospitalizations and infectious complications and indirectly by limiting the use of certain drug classes including immunosuppressants and antibiotics. CKD has been shown to increase mortality two- to fourfold amongst affected recipients. In the above mentioned analysis relative risk for death associated with CKD was 4.55 compared to the transplant recipient without CKD.

The glomerular filtration rate will tend to drop in the first few months after transplant with subsequent stabilization although a significant number will progress to ESRD. In one study the cumulative risk of ESRD was 4.7% and an annual ESRD risk of 1.5–2% [33].

The development of CKD in the organ transplant population is attributed to a number of risk factors, predominant amongst them being medication induced nephrotoxicity, pre-existing renal disease, and metabolic risk factors like diabetes, dyslipidemia, hypertension, and hepatitis [34]. Advancing age, cardiovascular disease, pulmonary hypertension, and episodes of acute renal failure have all been found to be risk factors for development of CKD in heart, lung, and liver transplant recipients. The perioperative factors causing acute renal injury may also factor into development of chronic kidney disease in the long run. The underlying disease like chronic hepatitis C in liver transplant recipients and immune mediated injury in connective tissue disease patients are additional CKD risk factors to be considered. Preexisting renal dysfunction has been shown to be a risk factor for CKD post-transplant. A decrease of 10 ml/min per 1.73 m in the pre-transplantation glomerular filtration rate was associated with an increase of 9% in the risk of chronic renal failure [32, 33]. However calcineurin inhibitors remain the most important cause of CKD in the SOT population.

Calcineurin inhibitors (CNIs) have become the mainstay of all immunosuppressive regimens in SOT recipients and carry an inevitable risk of renal toxicity by virtue of a number of mechanisms [35] at the cellular level. CNIs can cause acute renal failure due to afferent arteriolar vasoconstriction [36] mediated by chemical metabolites and sympathetic nerve activation and also a chronic nephrotoxicity mediated by angiotensin II [37]. CNIs have been associated with stimulation of renin with juxtaglomerular cellular hyperplasia and angiotensin II and aldosterone led renal vascular injury, micro thrombosis, and finally tubulointerstitial fibrosis and glomerulosclerosis manifesting as CKD [33]. These effects may be additionally mediated by transforming growth factor (TGF) beta 1 [38] and plasminogen activator inhibitor [39]. Local renal factors important for susceptibility to CNI nephrotoxicity include variability in P-glycoprotein and CYP3A4/5 expression or activity, older kidney age, salt depletion, the use of NSAIDs, and genetic polymorphisms in genes like TGF-beta and ACE [35].

Clinical features of CKD are similar to the general population although the use of serum creatinine as a marker for renal function may be misleading in a large proportion of this population who are malnourished and have a decreased muscle mass. The GFR usually stabilizes after an initial accelerated decline and drops at a slower rate thereafter. Proteinuria is usually not a major feature in view of the anti-proteinuric effects of CNIs and may portend development of focal glomerular sclerosis post-cardiac transplant, or IgA nephropathy or membranoproliferative glomerulonephritis (MPGN) post-liver transplantation [40].

Distal renal tubular acidosis type IV associated with hyperkalemia has been described in the setting of CNI use [41, 42]. Cyclosporine associated hemolytic uremic syndrome has been described in association with hemorrhage colitis [43].

A renal biopsy with features of striped atrophy and tubulointerstitial fibrosis with arteriolar hyalinization and glomeruli with sclerosis is consistent with chronic CNI toxicity [44] while as acute CNI toxicity has been associated with lysosomal vacuolization of tubular cytoplasm. Many times renal dysfunction may not have histological correlates being a predominantly functional vascular process [45].

The management of CKD in this population is similar to the general population with some caveats. Prevention and eventually therapy for CNI nephrotoxicity are aimed at lowering total systemic blood levels and renal exposure to the CNIs or their metabolites. The CNI-based immunosuppression may need to be adjusted to achieve lower levels as allowed by the risk of rejection of the allograft. mTOR agents like sirolimus have been used after the initial period of healing post-transplant to decrease the risk of CNI nephrotoxicity.

Calcium channel blockers mainly the dihydropyridine [46] and angiotensin inhibitors [47] have been postulated to block some vasoconstrictive effects and hence improve GFR but have not become the mainstay as the evidence of benefit at this point is not conclusive. Other potential therapies include vasodilatory prostanoids, L arginine, statins, spironolactone, and experimental treatments targeting the mediators like TGF-beta [35].

Optimal control of blood sugars and blood pressure and other cardiovascular risk factors needs to be undertaken.

Finally renal transplantation is an option for this group and has been shown to confer a survival benefit in non-renal transplant patients with ESRD [48]. After the initial transient post-operative increase in mortality, patients who received a kidney transplant had a lower risk of death, which was sustained until 5 years after transplantation [32].

53.4 Neoplastic Complications

The burden of malignancy in SOT recipients is related in part to de novo development of neoplasia and partly to the aggressive course of naturally occurring malignant tumors.

Historically the increase in incidence of malignancies in SOT recipients has run parallel to more robust and multipronged immunosuppression. A surge in the incidence of lymphomas with the introduction of polyclonal antibodies in 1960s and again with the use of cyclosporine in 1980s was a result of this effect [49].

The role of immunosuppression is also corroborated by the data from HIV-infected patients who demonstrate similar increases [50].

The various factors postulated to contribute to this increase in malignancies include oncogenic infectious agents like some viruses (Table 53-3), malignancies from the donor not identified at the time of transplant, direct effects of immunosuppression leading to potential sparing of aberrant cells with malignant potential, and some predisposition from underlying disease.

The spectrum of cancers occurring in this population is wide with an overall two- to fourfold increased risk of cancer compared to general population and an excess absolute risk of 0.7% according to a cohort study using the US Scientific Registry of Transplant Recipients (SRTR) with data on 175,732 solid organ transplant recipients [51].

Non-Hodgkin lymphoma and cancers corresponding to three commonly transplanted organs (kidney, liver, and lung) together comprised 43% of all cancer cases in transplant recipients compared with 21% in the US general population [51].

Risk was increased for 32 different malignancies, some related to known infections (e.g., genital and anal cancer related to human papillomavirus (HPV), Kaposi sarcoma due to human herpesvirus 8 (HHV-8), non-Hodgkin's lymphoma with Epstein–Barr Virus (EBV) and stomach cancer possibly with Helicobacter pylori) and others unrelated to infections (e.g., non-melanoma skin cancers, thyroid, colorectal, and lip cancers). Breast, prostate, ovarian, brain, and testicular cancers were not increased in incidence in this population which may point against an infectious contribution to these cancers [52].

Other population based cancer registries for transplant recipients have also reported similar findings. These include the increased non-Hodgkin's lymphoma and colorectal cancer risk reported in the Canadian liver transplant recipients [53]. Increased risk of head and neck cancers, lymphoma, leukemia, and lung cancer was reported in the Australian cardiothoracic organ transplant cohort [54].

Excess risk for non-melanoma skin cancer, non-Hodgkin's lymphoma, and stomach cancer was observed in Swedish organ recipients [55].

The incidence of specific malignancies does depend on the organ transplanted with two- to threefold higher frequency of non-Hodgkin's lymphoma and lung cancer in lung transplant recipients and more liver and kidney malignancies seen in liver and kidney recipients, respectively.

Variation in cancer risk between recipients of different organs is likely to arise from the degree of immunosuppression maintained in cardiothoracic recipients.

53.4.1 Post-Transplant Lymphoproliferative Disorder (PTLD)

Non-Hodgkin lymphoma is the second most common malignancy in SOT recipients after non-melanoma skin cancer. PTLD is a dreaded complication of organ transplant. It is one extreme of EBV driven monoclonal lymphoid proliferation on a spectrum at the other end of which is seen benign lymphoid hyperplasia with a mononucleosis type syndrome. The most common non-Hodgkin lymphoma subtype amongst both transplant recipients and HIV-infected individuals is diffuse large B cell lymphoma, and most cases have detectable EBV in tumor cells [56].

The pathogenesis of PTLD is hypothesized to be related to B cell proliferation driven by EBV expressing all latent antigens (Type 3 latency program) which remains unchecked because of the T cell suppression related to the immunosuppressive agents in transplant recipients [57]. EBV infected B cells are activated to transcribe proteins from genes like Latent Membrane protein (LMP) and EBNA (Epstein–Barr nuclear antigen). Normally the expression of these proteins leads to activation of the cytolytic T cells and keeps the EBV in check. However in SOT recipients the cytolytic T cell response is suppressed leading to unrestricted EBV invoked B cell proliferation. Other potential mechanisms include IL 10 related suppression of antiviral immunity and a decrease in precursor plasmacytoid dendritic cells which play a role in antiviral immunity [58].

In general, agents that suppress T cell activity appear to be associated with an increased risk of PTLD [59].

Bimodal onset of PTLD (Figure 53-3) following organ transplantation has been described previously [56] and risk factors differ somewhat for early-onset and late-onset PTLD, supporting etiological heterogeneity. Most of the cases were believed to originate from the recipient but further studies have revealed that donor-derived tumors can occur as well [60]. The origin may have implications on the time of disease occurrence and the prognosis. PTLD originating from the donor has been suggested to arise in the first year after transplantation into the graft, and recipient-origin PTLD develops later as an invasive disease and is more aggressive [61]. Early-onset PTLDs tend to be EBV-positive and, when extra nodal, are more likely than late-onset PTLDs to be localized to the transplanted organ. Late-onset PTLD is less likely to be associated with EBV and, overall, is more likely than early-onset PTLD to be extranodal [62].

Non-Hodgkin lymphoma risk was most pronounced amongst young transplant recipients, who are susceptible to primary EBV infection following transplantation and the highest risk was amongst lung recipients, possibly as a result of the high intensity of immunosuppression which needs to be maintained to prevent rejection. Another factor implicated is the larger amount of lymphoid tissue within the lung allograft [63].

The etiology of EBV-negative PTLD has not been fully elucidated, but gene expression profiling studies suggest that

TABLE 53-3. Known infectious associations of cancers in transplant recipients

Post-transplant lymphoproliferative disorder (PTLD)	EBV
Kaposi's sarcoma	HHV8
Anogenital cancers	HPV
Other rarer cancers, e.g., primary effusion lymphomas	HHV 8
Liver cancer	Hepatitis B and C virus

they are biologically distinct from EBV-positive disease. Some may be related to EBV infections that are no longer detectable. Others may be due to unidentified viruses or other causes of chronic antigenic stimulation. As an example, post-transplantation primary effusion lymphoma is associated with HHV-8 infection [64].

An association with CMV has been reported possibly reflecting the immunosuppressive effects of the virus [65].

A donor–recipient mismatch for EBV serostatus with reactivation or primary EBV infection increases the risk of PTLD. In one study, in the absence of the other risk factors, the incidence rate of PTLD for EBV-seronegative recipients was 24 times higher than that for EBV-seropositive recipients [66].

EBV DNA PCR in blood has been advocated as a screening tool in high risk patients and in those with symptoms. However it appears that peripheral EBV PCR may be below the detectable levels in some cases and in these intracellular EBV and other risk factors may play a role. Absence of EBV viremia should not be used to rule out PTLD. On the contrary, EBV DNAemia is very common in all transplant recipients, so that the positive predictive value of EBV DNAemia for predicting the development of PTLD is generally low [67].

The clinical presentation of patients with PTLD is highly variable and depends at least partially upon the type of PTLD and the site of involvement. Non-specific constitutional symptoms such as fever, weight loss, and fatigue are common. Other symptoms may be related to viral infection, lymphadenopathy, dysfunction of involved organs, or compression of surrounding structures. Viral symptoms can resemble those seen in acute infectious mononucleosis, although this is generally seen only in pediatric transplant recipients.

In most cases PTLD presents as a systemic disease involving lymphoid tissue, spleen, central nervous system, or another extra lymphoid tissue [65]. More than half of PTLD presents with extra nodal masses. Extra nodal organs include the gastrointestinal tract (stomach and intestine), lungs, skin, liver, central nervous system, and the allograft itself with symptoms referred to the organ involved. Central nervous system disease (which is rare in the general population) is found as well in about 20% and a similar proportion have infiltrative lesions in the allograft. Involvement of the allograft can lead to allograft dysfunction, including renal failure, heart failure, and respiratory dysfunction. The systemic manifestations may also include unexplained anemia, thrombocytopenia, or leukopenia, elevated level of serum lactate dehydrogenase (LDH), hypercalcemia, hyperuricemia, or monoclonal protein in the serum or urine.

PTLD is further classified according to the subtype of lymphoma. The vast majority of these tumors are B cell lymphomas, most common being diffuse large B cell lymphoma (DLBCL), and less common Burkitt's lymphoma or plasma cell neoplasm (e.g., myeloma or plasmacytoma).

Management of PTLD has varied significantly according to the type of lymphoproliferative disease present, as well as from institution to institution.

Immunosuppression reduction is the cornerstone of therapy. In addition since PTLD is a disorder of CD20 positive B cells effective therapies include rituximab which has become first line treatment. Rituximab a human/mouse chimeric anti-CD20 monoclonal antibody appears to have a survival benefit.

Patients who fail are treated with chemotherapy, radiation therapy, or a combination of these. Other treatments, such as adoptive immunotherapy with EBV specific cytotoxic T cells, are generally reserved for persistent disease despite initial therapy.

A preventive role for anti-EBV agents including ganciclovir and acyclovir has been explored. However at present evidence of long-term benefit appears to be lacking.

Kaposi's sarcoma (KS) is another tumor with increased frequency after organ transplantation. The true prevalence of this tumor depends on the geographic origin of the recipients, being higher in the Mediterranean basin and Africa. In the French GCIF registry a prevalence of 0.52% was seen with higher values in liver transplant recipients (1.24%) than the kidney and heart transplant recipients (0.45% and 0.41%, respectively). Other HHV-8 associated disorders in SOT recipients include multicentric Castleman disease, primary effusion lymphoma, and HHV-8 associated inflammatory cytokine syndrome. Of note, HHV-8 infection can be donor derived; screening for HHV-8 in donor and recipients is not currently recommended because of the poor specificity of current available assays [68, 69].

53.4.2 Skin Cancers

Non-melanoma skin cancers are the most frequent types of cancers seen in post-organ transplant. Out of these squamous cell cancer is the more common pathologic diagnosis with an incidence 65 times the general population followed by basal cell with 10 times the incidence compared to the general population [70].

These tumors are also more aggressive with increased risk of metastases and recurrence. The risk factors for skin can-

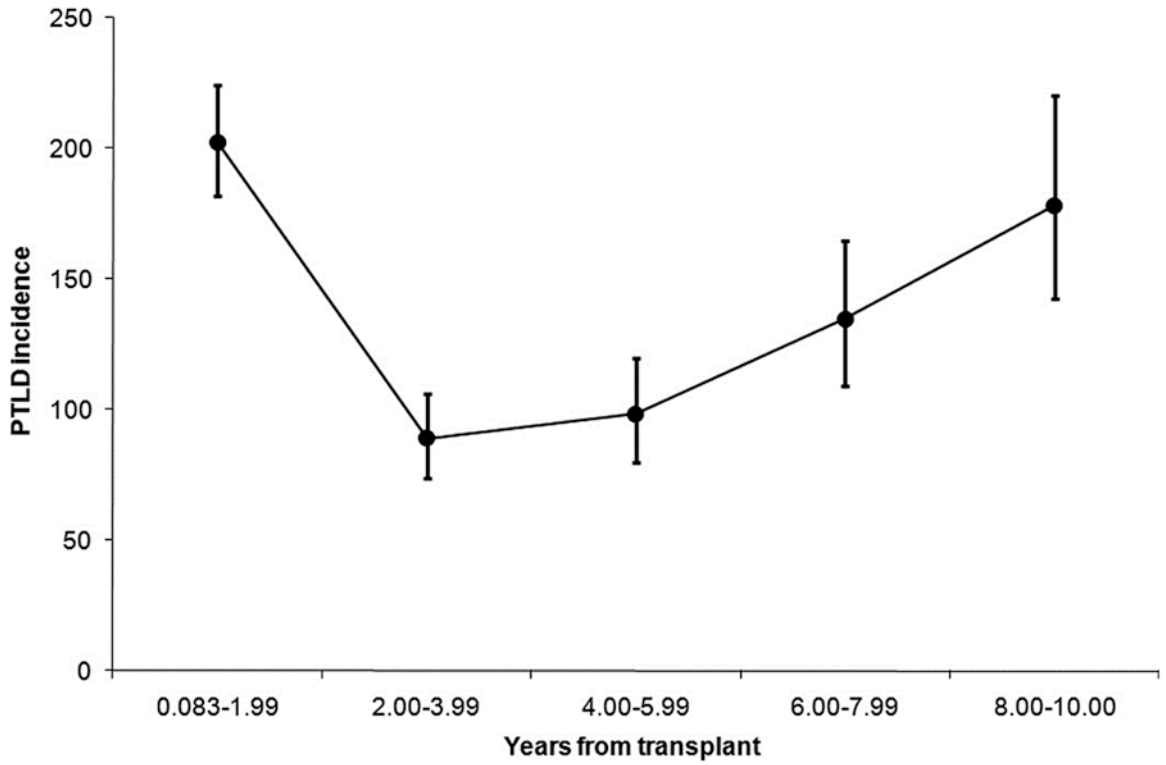

FIGURE 53-3. Bimodal pattern of PTLD incidence in kidney transplant recipients. From Quinlan SC, Pfeiffer RM, Morton LM. Risk factors for early-onset and late-onset post-transplant. lymphoproliferative disorder in kidney recipients in the United States. Am J Hematol. 2011 Feb;86(2):206–9.

cers include Fitzpatrick skin type I to III, photosensitivity, cumulative sun exposure, increasing age, and increased intensity and duration of immunosuppression [71].

The disease is predominantly seen in fair skinned patients and in the sun exposed sites, e.g., head and neck area and the hands. These patients need to have a skin examination before transplant and then follow-up regularly.

The risk of developing skin cancers is higher in lung and heart–lung transplant recipients followed by kidney transplant recipients with the lowest risk seen in liver transplant recipients. This is likely due to lower levels of maintenance immunosuppression in this population and the age at transplantation [72].

There is some data hypothetically linking human papilloma virus (HPV) to the increased incidence of skin cancers but a causal relation is not proven. SOT recipients have been shown to harbor greater quantities of some HPV types in squamous cell cancers and E6/7 transcripts of HPVs 8, 9, and 15 have been found in some squamous cell cancers and predisposing actinic keratosis suggesting a role in pathogenesis [73, 74]. Of note, the impact of HPV vaccination in reducing the incidence of de novo skin cancer after transplantation has not been investigated.

The risk of less common skin cancers like melanomas and Merkel cell carcinoma is also increased in organ transplant recipients [71]. There is a three- to fivefold increased risk of melanomas in the transplant recipients [75]. US Scientific Registry of Transplant Recipients study revealed a standardized incidence ratio (observed/expected cases) of 2.38 [51]. The mean duration to development of melanoma after transplant is 5 years [75]. The risk factors for development of melanoma are multiple nevi, degree of immunosuppression, and fair complexion.

Voriconazole by virtue of causing photosensitivity has been associated with predisposition to development of skin cancers in the SOT recipients. This data comes from the lung transplant population which has the highest incidence of invasive aspergillosis amongst solid organ transplant recipients [76] requiring the use of voriconazole.

The type of immunosuppression is also related to the incidence of skin cancer. Azathioprine has been known to have photosensitizing effects by virtue of its metabolite 6-thioguanine which may result in photocarcinogenesis. The potential etiology could be by formation of highly reactive oxygen radicals [77] and replacing azathioprine has been shown to decrease skin photosensitivity [78].

The anti-angiogenesis and anti-proliferative properties of sirolimus have been hypothesized to be associated with a reduced incidence of malignancy post-transplantation [79]. Conversion to sirolimus based regimen after 6 months of transplant in selected high risk patients with stable graft function may have some value [80, 81].

We suggest regular cancer screening for solid organ transplant recipients in accordance with the age appropriate guidelines for general population with some caveats (Table 53-4). The benefits of screening are likely to be high in the case of malignancies that are noted to be increased after solid organ transplantation. However, more frequent screening may also lead to false positive results and unnecessary further testing. This approach should only be considered as a general guidance since no studies have formally evaluated the value of cancer screening in the transplant population.

53.5 Hypertension (HTN)

A significant proportion of solid organ transplant recipients develop hypertension after transplant with the prevalence ranging from 50% to 80% [82]. The risk factors for this include immunosuppressive treatment mainly the CNIs. The other contributing factors include the use of steroids, chronic kidney disease, weight gain related to steroids, and other factors.

A variety of mechanisms have been postulated to explain CNI related HTN. These include endothelial dysfunction with imbalance between vasoconstrictor endothelin [83] and vasodilator substances like prostacyclin, reduced GFR with increased sodium reabsorption [84], renin angiotensin aldosterone level increases particularly later in the course [85], and possible sympathetic activation.

HTN should be aggressively managed in this population according to the usual guidelines. Anti-hypertensive agents should be used as needed with monitoring of renal function and counseling about weight loss strategies and other metabolic risk factors. There may be some use of changing cyclosporine to tacrolimus or decreasing the target levels of CNI as allowed by the allograft function if the HTN is recalcitrant (http://m.srtr.org/2011/chapters/lung/images/12%20LU%20s5%20fig%207-01.png).

53.6 Diabetes Mellitus

Ten to fifteen percent organ transplant recipients develop de novo DM post-transplant. Some studies have shown that the cumulative incidence of this condition in heart transplant recipients may reach 32% at 5 years [86] similar to that reported in kidney and liver transplant patients. The 12-month cumulative incidences of new-onset diabetes after transplantation were found to be 13% for heart, 11.4–11.9% for liver, and 1.8–21.7% for kidney transplantation [87].

This is mainly related to the mainstays of immunosuppression which are CNIs and steroids. In addition underlying factors like age, family history, and obesity play a role as well. CNIs are diabetogenic by decreasing the secretion due to pancreatic β-cell apoptosis and synthesis of insulin and possibly calcineurin related insulin resistance [88].

Steroids cause DM via multiple mechanisms including insulin resistance, weight gain, and increased appetite. Most of these effects are pronounced in the first year of transplantation when the doses of these medications are quite high [89, 90].

53.7 Hyperlipidemia and Cardiovascular Disease

Hyperlipidemia is common after SOT and about half of the organ recipients develop some form of dyslipidemia with the incidence of hypertriglyceridemia and hypercholesterolemia after liver transplant reported as being over 60% [91]. The main factors predisposing to this are the immunosuppressant meds including steroids, mTOR inhibitors, and CNIs and other comorbid conditions like diabetes and obesity, diet and genetic predisposition. It has been suggested that cyclosporine may inhibit the enzyme 26-hydroxylase thereby decreasing the synthesis of bile acids from cholesterol and subsequently the transport of cholesterol to the intestines. Cyclosporine is also reported to bind to the low-density lipoprotein (LDL) receptor, which results in increased serum levels of LDL cholesterol [92]. Corticosteroids are reported to contribute to hyperlipidemia via several mechanisms. These include enhancing the activity of acetyl-coenzyme A carboxylase and free fatty acid synthetase, increasing hepatic synthesis of VLDL, down-regulating LDL receptor activity, increasing the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, and inhibiting lipoprotein lipase [93].

Hypercholesterolemia in organ transplant recipients may be associated with an increased risk for allograft vasculopathy. In cardiac transplant recipients, transplant vasculopathy takes the form of transplant coronary artery disease (CAD); in renal transplant recipients, vasculopathy manifests as chronic rejection; in hepatic transplantation, vascular involvement of biliary epithelial blood supply can manifest as vanishing bile duct syndrome. Atherosclerotic vascular disease in non-transplant vessels is another possible consequence. It has been reported that 55% of deaths in renal transplant recipients with functioning allografts were cardiovascular related. Peripheral vascular disease has been reported in 10% of heart transplant recipient [94, 95].

Cardiovascular disease (CVD) is a common cause of morbidity and mortality after solid organ transplantation. Both preexisting cardiovascular risk factors in recipients and immunosuppressive drug toxicity may contribute to CVD. In a study of 126 patients by 3 years after transplantation, 90% of patients had developed at least 1 cardiovascular risk factor and 40% developed ≥2 risk factors. The cumulative prevalence of new-onset hypertension at 1, 3, 5, and 7 years was 45%, 65%, 67%, and 72%, respectively. The corresponding

Table 53-4. Suggested screening for malignancies in organ transplant recipients

Organ	Recommendation
Skin	Monthly self-examination with a yearly skin examination by specialist. Early resection of suspicious lesions
PTLD	Periodic clinical assessment for symptoms or signs. In EBV mismatched patients viral titers in blood/plasma have been followed early post-transplant
Breast	Periodic self-examination and annual age appropriate mammograms
Colorectal	Colonoscopy every 10 years above 50 years of age. Earlier and more frequent if high risk. Fecal occult blood yearly but less specific
Lung	Yearly low dose CT in patients with native lung and over 30-pack year smoking. Need to consider alternate etiologies for findings
Cervical	Annual pelvic examination and pap smear for >18-year-old females. Role of HPV DNA testing unclear
Renal and urogenital	Low threshold for imaging (annual ultrasound) with high risk factors including analgesic abuse, cytotoxic chemotherapy, multicystic renal disease, and hematuria
Hepatocellular	Consider periodic screening with ultrasound and alpha fetoprotein if recipient is hepatitis B or C infected
Prostate	Consider yearly PSA and digital examination for patients over 50 years

prevalence for hypercholesterolemia was 16%, 33%, 48%, and 58%, and for diabetes 6%, 7%, 7%, and 10%, respectively, [96].

53.8 Bone Disease

Bone loss after SOT is a common and significant problem and can develop within months of transplantation [97]. Osteoporosis develops in a significant proportion of SOT recipients. The prevalence of osteopenia and osteoporosis can reach up to 50% in renal transplant recipients and is associated with increased incidence of fractures. 25% of liver and kidney transplant recipients develop pathological fractures. Osteoporosis at the lumbar spine (LS) has been reported in approximately 28% and at the FN in approximately 20% in cardiac transplant and up to 73% in lung transplant recipients [98] with fracture rates from 18% to 65% most commonly effecting the spine and ribs.

The bone loss is related to several pre- and post-transplantation factors including steroid use and dose, renal disease and osteodystrophy, vitamin D disorders, lack of exercise, hypogonadism, parathyroid hormone levels, malnutrition, older age, and female gender are other predisposing factors. Cyclosporine may be associated with bone loss by direct effects on calcineurin genes in osteoclasts [99] or indirectly via T cell mediated effects. Rapid bone loss with FK506 has been reported in both cardiac [100] and liver [101] transplant recipients.

There is some data that patients with osteoporosis or osteopenia showed worse graft function at 1 and 8 years compared with patients with normal bone mass density [102]. The most rapid rate of osteoporosis occurs during the first year of transplantation [103] most likely due to higher doses of immunosuppression, less weight bearing, and higher metabolic and other comorbid disturbances.

Careful monitoring of patients with bone densitometry both pre-transplant and periodically post-transplant and use of calcium and Vit D supplements along with resistance training and exercise is very important in risk assessment and in preventing and treating bone loss and pathological fractures. The management focuses on prevention by modifying the risk factors outlined above and treatment as indicated.

Bisphosphonates are the treatment of choice for preventing transplant bone loss particularly in patients with evidence of bone loss, history of fragility fractures, and other risk factors. Calcitriol is an alternate agent for preventing bone loss.

53.9 Obesity in Organ Transplant Patients

Obesity is a significant problem in the organ transplant population and in the setting of other cardiovascular risk factors common in this group, including hypertension and diabetes, can have deleterious consequences. The use of steroids and other immunosuppressive medications predisposes to obesity and other cardiovascular issues. An accelerated weight gain of about 20 bounds has been described within the first year of transplant in liver, heart, and kidney recipients [104–106].

The rate of post-transplant metabolic syndrome in liver transplant recipients is more than twice that reported for the general population and has been associated with cardiovascular morbidity [107]. Obesity has also been recognized as a risk factor for post-transplant fatty liver [108]. Metabolic syndrome has also been noted to be more common in kidney recipients and associated with coronary artery disease [109]. Cardiac transplant recipients with BMI > 35 at the time of transplant had significantly higher morbidity and mortality [110]. Obesity is recognized as an independent risk factor for primary graft dysfunction after lung transplantation [111]. Obesity has also been observed to be a risk factor for death after lung transplantation [112].

As the long-term survival from organ transplant increases it is conceivable that the consequences of obesity and the metabolic syndrome will become more noticeable and important to address.

53.10 Chronic Allograft Dysfunction

Chronic allograft dysfunction has been a significant long-term issue in organ transplant recipients not succumbing to other processes. It is known by different names depending on the organ: chronic allograft nephropathy (CAN), transplant glomerulopathy, or chronic renal allograft dysfunction in kidney transplant recipients; chronic allograft vasculopathy (CAV) in heart transplant recipients; vanishing bile duct syndrome (VBDS) in liver transplant recipients, and bronchiolitis obliterans syndrome or chronic lung allograft dysfunction (CLAD) in lung transplant recipients.

Chronic allograft dysfunction points to a variable and usually slow decline in the organ function beginning within months of transplant. It is difficult to differentiate this process from other contributing processes or diseases and usually is a diagnosis of exclusion with some support provided by histopathology from a biopsy. The clinical significance of chronic allograft dysfunction depends also on the organ transplanted. While CLAD is the main cause of allograft loss and death after lung transplantation (50% at 5 years), the incidence and outcomes of VBDS in liver transplant recipients have not completely been established.

The histopathology of CAN is characterized by fibrous intimal thickening of arteries, glomerulosclerosis, interstitial fibrosis, and tubular atrophy [113]. It is common at 10 years after transplantation being present in over 50% of patients [114]. It is manifested by decline in kidney function, hypertension, and proteinuria [115].

The pathophysiology of this process is unknown and is most likely multifactorial with contributions from immunological factors like antibody-mediated rejection and non-immunologic factors. Drug toxicity, bacterial or viral infection, hypertension, obstruction, recurrent or de novo renal diseases, and acute and chronic cell- and/or antibody-mediated rejection need to be addressed and some cases remain as "interstitial fibrosis and tubular atrophy, no specific cause (IFTA)" [116].

Infection significantly contributes to the development of chronic allograft dysfunction in kidney transplant recipients, and in particular in case of viral infections (Figure 53-4). The mechanisms as to why infection contributes in the impairment of allograft function include, on the one hand, the development of direct allograft injury (for example, in case of BK virus associated nephropathy or by inflammation associated with CMV disease or by bacterial urinary tract infection) and, on the other hand, by immunological factors triggered by viral replication, such as the activation of the innate and adaptive immune systems, leading to the development of acute and chronic rejection. This has particularly been observed after CMV infection [117]. The impact of CMV replication in impairing allograft outcomes has significantly been reduced with the introduction of universal preventive strategies against CMV, mainly with the use of antiviral prophylaxis, which has been associated with a decrease in the rate of acute rejection [118] and graft loss [119]. However, studies assessing long-term outcomes (i.e., > 10 years) in kidney transplant recipients receiving antiviral prophylaxis are lacking.

Similarly in the lung transplant recipients a majority of recipients develop a relentless dysfunction to varying degrees called CLAD which encompasses the commonly recognized bronchiolitis obliterans syndrome (BOS) an obstructive process and other recently recognized processes including restrictive allograft dysfunction (R-CLAD) and a neutrophilic variant which is azithromycin responsive allograft dysfunction (ARAD) [120]. The pathophysiology of these processes remains obscure. Recurrence of some native disease processes like sarcoidosis needs to be ruled out. Infection appears to play an important role in the development of CLAD after lung transplantation. In addition to CMV, other viral infections such as influenza and RSV infection have been associated with both transient and progressive decline in lung allograft function. Influenza vaccination may reduce the burden of influenza-associated allograft dysfunction. Also, some studies have identified de novo colonization of the respiratory tract with *Pseudomonas aeruginosa* [121] and colonization/infection with *Aspergillus fumigatus* [122] as significant risk factors for the development of CLAD. The role of antibiotic and antifungal prophylaxis in reducing the incidence of CLAD has not yet been established.

In heart transplantation, this disease process is referred to as cardiac allograft vasculopathy (CAV) contributed to by immunologic factors and non-immunologic processes like hyperlipidemia, cytomegalovirus infection [123], baseline coronary artery disease, and the mechanism of brain death in the donor [124]. CAV affects over 30% of patients within 5 years of cardiac transplantation [125].

Histological abnormalities are commonly present in late post-transplant biopsies, including protocol biopsies from patients who appear to be well with good graft function. Recurrent disease is the commonest recognized cause of abnormal graft histology, but may be modified by the effects of immunosuppression or interactions with other graft complications, resulting in complex or atypical changes [126].

53.11 Neurotoxicity

CNIs are associated with neurotoxicity in a significant number of organ recipients. The manifestations of this toxicity lie along a wide spectrum with the most common manifestation being tremor related to tacrolimus or cyclosporine. This tremor may range from mild to disabling in which case a change in immunosuppression may be warranted. CNIs also have other minor non-specific CNS effects including

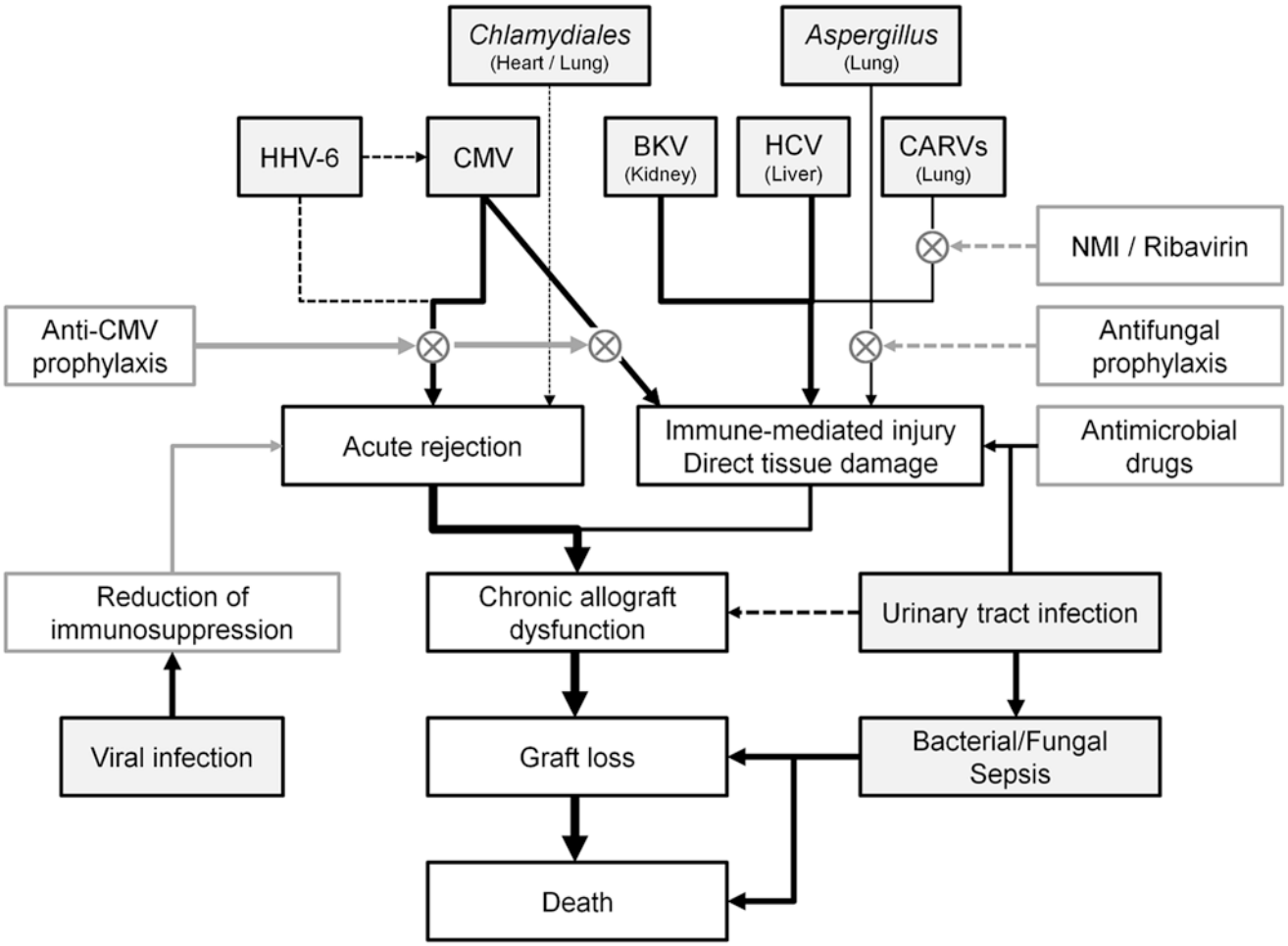

FIGURE 53-4. Potential interaction between infections and transplant outcomes. From Martin-Gandul, C., Mueller, N. J., Pascual, M. and Manuel, O. (2015), The Impact of Infection on Chronic Allograft Dysfunction and Allograft Survival After Solid Organ Transplantation. American Journal of Transplantation. doi: 10.1111/ajt.13486.

headaches, confusion, mild encephalopathy [127], memory issues, and insomnia or major complications, as neuropathy, reversible posterior leukoencephalopathy syndrome, and seizures. Some of the effects have been related to the hypomagnesemia and hypertension related to tacrolimus.

These effects are usually seen early on after initiation but can happen after long-term immunosuppression [128].

Beta blockers and primidone have been used for the tremor if stopping the medication is not an option.

53.12 Hematological Toxicity

The common effects include anemia, leukopenia, and thrombocytopenia. The factors predisposing to this include medication effects on bone marrow from drugs like mycophenolate and sirolimus used for immunosuppression, valganciclovir or acyclovir used for treatment or prophylaxis of CMV or HSV, and trimethoprim and sulfamethoxazole used as prophylaxis for *Nocardia* and PCP. In cases of liver transplant some degree of pre-transplant hypersplenism may contribute to this issue.

53.13 Psychiatric Complications

The process of organ transplantation takes a toll on the recipient psychologically with anxiety and depression being common problems after surgery. The effects of the intensive care stay, the need for an intensive post-transplant management, fear of complications, and social and economic impact require a psychological resilience and coping that can lead maladjustment. About 20–63% organ transplant recipients develop anxiety or depressive disorders post-transplant [129]. The number seems to be higher for cardiothoracic transplants.

53.14 Conclusion

In general as the life expectancy of solid organ transplant recipients improves, the care of these patients needs to be all encompassing addressing community-acquired viral and bacterial infections, metabolic and cardiovascular complications, and a wider range of neoplasia with a focus on preventive maintenance as well. The periodic screening of this population for the late complications discussed will likely have an impact on morbidity and outcomes.

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Erratum to: Special Considerations for Long-Term Survivors After Solid Organ Transplantation

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The original version of this chapter Table 53-2 had the wrong headings--duplicated one year events. This has been corrected and the correct heading has been updated to Five year events 2004-06 tx in the last column.

The updated online version of this chapter can be found at
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