Cytomegalovirus and Epstein-Barr Virus Infection and Disease

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Key Points

- PTLD is a potentially fatal but preventable complication in paediatric liver transplant recipients, often related to EBV.
- In PTLD, stratifying the risk according to clinical and histological features and to response to immunosuppression withdrawal helps identifying the subset of patients who should benefit from rituximab and the few patients needing chemotherapy.
- CMV disease after liver transplantation can be effectively prevented by preemptive therapy, with reduced costs and antiviral exposure.
- CMV resistance should be suspected in patients not responding to 2 weeks of ganciclovir treatment and assessed through genotypic resistance testing.

Research Needed in the Field

- To find tools allowing to better understand the degree of immune competence of each patient during standard immunosuppressive treatment and tailor it to prevent severe viral infections
- To find strategies boosting the immune system and promoting the clearance of EBV infection, when occurred
- To identify prognostic factors defining patients with PTLD without satisfactory response to rituximab that benefit from the addition of cytoreductive chemotherapy
- To improve the monitoring of CMV infection and find new antiviral treatments efficacious on mutants resistant to ganciclovir

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34.1 Introduction

Currently, the greatest rate of mortality following paediatric liver transplantation (LT) is related to infections, certainly related to excessive immunosuppression [1]. The impact of viral disease on LT outcome depends mainly on two factors: firstly, the time post-LT, thus the degree of immunosuppression, and secondly the serological status of the recipient. Most of the infectious complications following LT are caused by herpes viruses and are acquired with the donor organ [2]. Indeed, children are often seronegative recipients receiving livers from adult donors latently infected by cytomegalovirus (CMV), Epstein-Barr virus (EBV, and, less frequently, herpes simplex virus (HSV1-2) and human herpes virus 6 and 8 (HHV6, HHV8). Under maximum immunosuppression, this often leads to early infection/reactivation of the viruses, with different consequences.

In this chapter, the role of CMV and EBV, the main donor-associated viral pathogens, will be discussed.

34.2 EBV Infection and PTLD

34.2.1 Clinical Pictures and Epidemiology

EBV is responsible for a spectrum of clinical conditions that depend on the level of lymphoid tissue involvement and transformation, resulting from the interaction between the virus and the immune system. About 60–80% of seronegative children are expected to acquire EBV infection within 3 months of solid organ transplant, either from primary oropharyngeal EBV infection or via donor passenger lymphocytes in the transplanted organ from a seropositive donor.

Symptomatic EBV infection occurs in 8–22% of the cases and is not different from that of the immunocompetent host [3, 4]. It is commonly defined in the presence of IgM against the viral capsid (or positive viral load) along with either the

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histological evidence of an EBV infection or specific symptoms (fever, leukopenia, atypical lymphocytosis, exudative tonsillitis and/or lymphadenopathy, or hepatitis).

The most important and potentially fatal complication related to EBV is the post-transplant lymphoproliferative disorder (PTLD), which is defined on the basis of the histological criteria. PTLD represents a continuum of atypical lymphoid proliferations, ranging from lymphoid hyperplasia to malignant lymphomas, typically but not exclusively of B-cell origin.

The overall reported incidence of PTLD varies between 3 and 18%, with a mortality rate previously peaking at 60%, but nowadays ranging between 10 and 12% [3, 5–8].

In the North American centres of the SPLIT Consortium, comparing the periods 2002–2007 and 1995–2001, the incidence of symptomatic EBV infection and PTLD decreased from 11.3% to 5.9% and from 4.2% to 1.7%, respectively. This reduction seems to be related to an increased attention to immunosuppression, with most centres maintaining lower trough levels of both cyclosporine A and tacrolimus in the first months after LT [4].

Acknowledged risk factors for developing PTLD are the EBV seronegativity at LT, young recipient age, older donors, high levels of immunosuppression and the use of lymphocyte-depleting agents [3, 4, 9].

The majority of PTLDs in children occur within the first 2 years after LT (early PTLD), and this is related to EBV acquisition and intensive T-cell suppression [10, 11]. It can be said that 90% of childhood PTLDs are EBV-related and early-onset. Late-onset PTLD can occur in paediatric LT recipients as a consequence of lifelong immunosuppression and represents less than 10% of cases. Late forms present more frequently as disseminated, often monomorphic, disease, sometimes EBV-negative. Late PTLD tends to be less responsive to the sole immunosuppression reduction, displaying a worse clinical course. In a study carried out by our group, it has been shown that late PTLD seems to be related to sustained, long-term EBV detection in blood, although at low viral loads. The study concluded that long-term monitoring of EBV-PCR until negativisation is recommended to prevent or promptly diagnose late PTLD [12].

The current World Health Organization (WHO) classification recognises four types of PTLD:

- *Early lesions:* almost invariably represent the histologic picture of the early PTLD, with reactive infectious mononucleosis-like plasmacytic hyperplasia, always EBV-positive, occurring at the time of primary immunoconversion.
- *Polymorphic PTLD:* defined by the concomitant presence of monoclonal EBV-positive B lymphocytes and polyclonal T cells.
- *Monomorphic PTLD:* this includes different subtypes. The vast majority are of B-cell origin and have large similarities with diffuse large B-cell lymphoma (DLBCL-like or PT-DLBCL). Burkitt and Burkitt-like lymphomas are less common, while the plasmablast/immunoblast lymphoma is exceptional.
- A fourth type encompasses Hodgkin's-like lymphoma, plasmacytoma-like PTLD and T-cell neoplasms.

Table 34.1 Classification system for post-transplant lymphoproliferative disorder (PTLD)

Grading	Description
0	EBV lymphadenitis, hepatitis, not classified as PTLD
1	Early lesion, low-grade mononucleosis, plasma cell hyperplasia
2	Polymorphic, diffuse B-cell hyperplasia (PDBH) and polymorphic B-cell lymphoma (PBC)
3	Monomorphic or lymphomatous PTLD or lymphoma, immunoblastic lymphoma (IBL), diffuse large B-cell lymphoma (DLBCL) or diffuse small cell noncleaved (Burkitt-like)
4	Other Hodgkin's-like PTLD, plasma cell lesions, plasmacytoma, T-cell PTLD

Adapted from Harris NL et al.

The classification of PTLD lesions is summarised in Table 34.1. This classification has important implications for treatment, allowing a risk-adapted management of PTLD that—along with a high index of suspicion and surveillance—is the most effective strategy to reduce PTLD lethality.

Due to its importance in the EBV burden after solid organ transplantation, the following sections will discuss PTLD.

34.2.2 Pathophysiology of PTLD

Following EBV infection, the immunocompetent host develops a cytotoxic T-lymphocyte response to the viral proteins exposed on EBV-infected B lymphocytes [13]. After seroconversion, a lytic response leads to viral clearance, followed by a memory T-cell response. In the immunosuppressed patient failing to clear the infection, the EBV is capable of promoting the germinal centre T-cell-dependent pathway and starting different types of latency, resulting in a variable degree of resistance to apoptosis. This results in the proliferative stimulus to the activated B-blast (mainly mediated by LMP1 and EBNA2 expression) but also in the latent infection of the germinal centre blasts, in which EBV latency proteins lead to a failure in deleting low-affinity B-cell receptor clones that become immortalised. The resulting cells become differentiated mainly into memory B cells but also into plasma cells. The latter allow viral lytic replication, which is important for lymphomagenesis, especially for the early stages [14, 15].

Different EBV proteins are involved in the complex mechanism of PTLD, from B-cell hyperplasia to malignancy. LMP1 is analogous to CD40 and promotes cell transformation by inducing the NF-kB pathway, with consequent upregulation of BCL-2 and other genes involved in blocking apoptosis; nuclear EBNA1 and EBNA2 warrant viral episomal DNA replication and selective cellular and viral gene expression, respectively; LMP2 acts as a chronically active B-cell receptor and provides further survival signals [16].

The most common malignant PTLD subtype is the diffuse large B-cell lymphoma (PT-DLBCL), followed by Burkitt lymphoma (PT-BL) and plasmablastic lymphoma (PT-PBL). EBV-driven disruption of B-cell maturation is displayed in Fig. 34.1. However, around 10% of EBV-negative and a few T-cell origin post-transplant lymphomas have a different pathogenesis. Mechanisms that contribute to both EBV-positive and EBV-negative lymphomas are T-cell suppression, microsatellite instability, epigenetic alterations (such as hypermethylation and aberrant up- or downregulation of host microRNAs) and host polymorphisms in genes related to immune response.

34.2.3 Diagnosis

Since symptoms can be non-specific, the diagnosis of PTLD relies on a high index of suspicion. The lymphoid tissue involvement of diverse organs accounts for the heterogeneity of localisations and clinical signs, ranging from pharyngitis and/or Waldeyer ring enlargement to gastrointestinal symptoms.

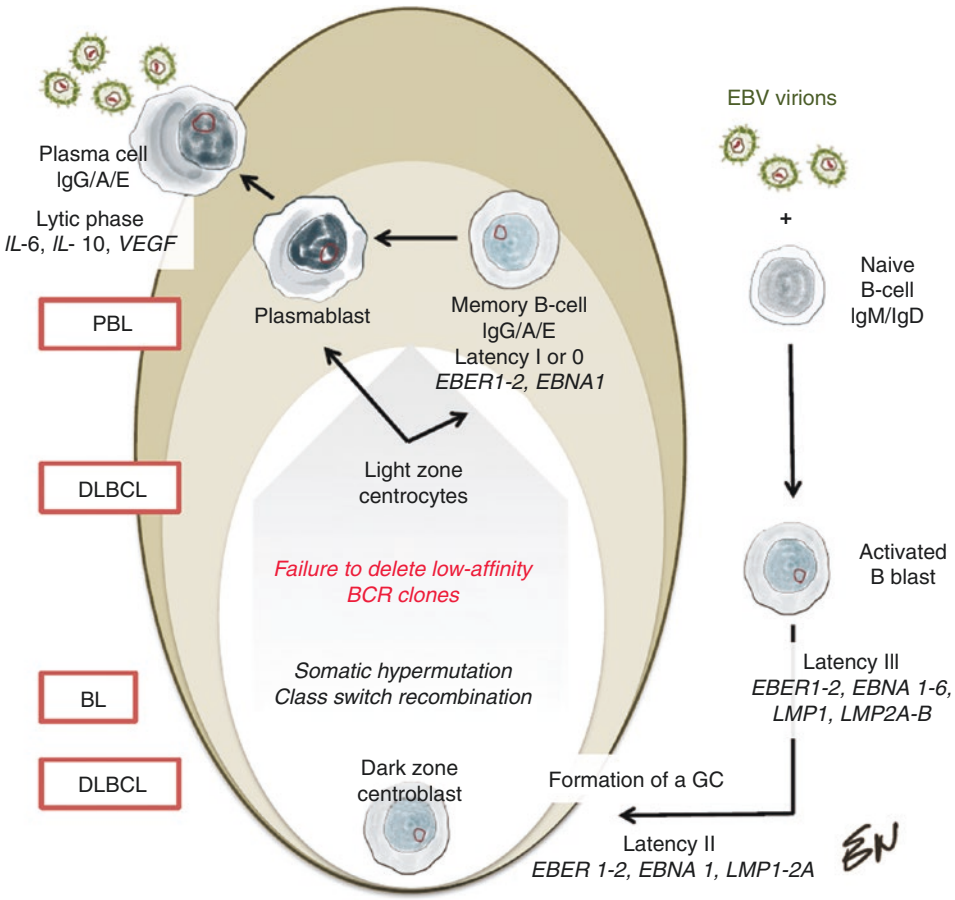

Fig. 34.1 Pathogenesis of the Epstein-Barr virus-mediated hyperplasia and malignancy in post-transplant lymphoproliferative disorder (PTLD). EBV exploits the normal B-cell activation pathway but succeeds to induce proliferation due to protein expression patterns. The activated EBV-infected blasts that enter the germinal centre (GC) express viral latency III pattern (LMP1+/EBNA2+), undergoing proliferation. Likewise, in the GC, latency II (LMP1+/EBNA2−) pattern is expressed by infected centroblasts undergoing somatic hypermutation and class switch recombination for antibody maturation. The selected B cells differentiate into plasma cells (that provide lytic phase that perpetuates the cycle) or memory cells (latency I, EBNA1+ or latency 0, no expression of viral proteins). The failure to control the EBV-associated proliferative stimulus by the immune system results in a spectrum of dysplastic and malignant B-cell subtypes that have features of their normal counterpart. *EBV* Epstein-Barr virus, *GC* germinal centre, *BCR* B-cell receptor, *DLBCL* diffuse large B-cell lymphoma, *BL* Burkitt lymphoma, *PBL* plasmablastic lymphoma. Adapted from [16]

Along with the clinical clues, the availability of viral nucleic acid monitoring has become a great tool to manage this complication. The viral load (measured in PBMC or in whole blood) is reliably correlated with the risk of developing symptomatic EBV infection and PTLD [17, 18]. However, EBV viral load, while useful to monitor EBV infection, is unable to reliably support the diagnosis of PTLD. Indeed, high viremia can be detected in the absence of PTLD, while, conversely, PTLD can be associated with low or even undetectable EBV viral loads, especially when it occurs in protected sites such as the graft itself or the gut. Furthermore, rather than the viremia per se, a more specific immune response conferring a substantial risk of developing PTLD is the high viral replication associated with a low number of anti-EBV cytotoxic T lymphocytes [19, 20].

The symptoms heralding PTLD are summarised in Table 34.2. The clinical presentation is often subtle and non-specific, with typically no or little involvement of peripheral lymph nodal stations. PTLD should be suspected in the case of otherwise unexplained fever, malaise or failure to thrive in a transplanted child, especially in the first year after LT or under augmented immunosuppression. Children with high or rapidly increasing EBV viral load, in the presence of physical signs or cytopenia, should also be considered at high risk of having PTLD. Clinical signs can be a mononucleosis-like syndrome, abdominal pain with or without diarrhoea or neurologic symptoms.

Imaging has a paramount role in confirming the suspicion of PTLD or staging a confirmed disease. Ultrasound has a limited role, while a neck, chest and/or abdominal CT or MR scan should be used as first-line tools as soon as the suspicion arises, since they can identify occult lesions amenable to tissue sampling.

In fact, histology is the pillar of the diagnosis of PTLD. Beyond the bulky lesions, histological evidence of the disease can be obtained from intestinal biopsies (early gastrointestinal endoscopy should be performed in case of even mild gastrointestinal symptoms or hypoalbuminemia due to protein-losing enteropathy) or from the graft, if evoked by specific clues. Histological analysis should always encompass in situ hybridisation to detect EBV-encoded small RNA (EBER), to confirm the disease is EBV-related, and CD3/CD20 stain for disease subtyping.

Table 34.2 Clinical signs of post-transplant lymphoproliferative disorder (PTLD)

Systemic symptoms	Unexplained fever or night sweats
	Malaise
	Weight loss
	Sore throat
	Headache or focal neurologic symptoms
General and organ-specific signs	Pallor
	Lymphadenomegaly
	Tonsillar enlargement
	Focal neurologic signs
	Mass lesions
	Subcutaneous nodules
	Diarrhoea, abdominal pain, gastrointestinal bleeding
	Nausea and vomiting
	Hepatosplenomegaly
	Jaundice, graft dysfunction

34.2.4 Treatment Algorithm

The basis of the treatment of PTLD are firstly restoring the cytotoxic T-lymphocyte immune response against the EBV and secondly targeting B-lymphocyte proliferation.

As for the first point, reduction or weaning of the immunosuppression is of great importance in controlling the lymphoproliferative modifications. This intervention is the gold standard for the early (usually polyclonal) PTLD, where it helps avoiding unnecessary medications, though exposing the patient to increased risk of rejection. The proportion of adult patients undergoing complete remission with this modulation alone shows a variation from 23 to 86% [21], reflecting the heterogeneity of the disease. Patients with monomorphic disease, or those having a poorer prognosis for disease extent or worse general conditions, are assigned to a more aggressive treatment.

Strategies to counteract B-cell proliferation are mainly based on the use of anti-CD20 antibodies and of cytoreductive chemotherapy regimens. Since the early 2000s, the availability of the humanised, chimeric anti-CD20 antibody *rituximab* has revolutionised the treatment of PTLD. Seventy percent of children who had an haematopoietic stem cell transplant (HSCT) and 64–84% of children undergoing a solid organ transplantation have prolonged disease-free survival after rituximab therapy, with better outcomes when this treatment is associated with immunosuppression reduction [22].

The association of rituximab with different low-dose chemotherapy regimens has been reported to warrant more robust results in children with PTLD, with an overall survival rate of between 83 and 86% and a 2-year event-free survival of 67–71% [11]. On the other hand, adding even a minimal cytoreductive regimen to rituximab leads to a non-negligible toxicity, essentially related to infections: chemotherapy-related mortality was 5–6% in two paediatric trials [11] and 11% in an adult trial [23], while it can be up to 50% for more intensive protocols [24, 25].

Thus it is important to identify prognostic factors defining patients not responding to rituximab that benefit from the addition of cytoreductive chemotherapy. Only few studies have translated this concern into a step-by-step approach to paediatric PTLD. In the non-randomised, response-adapted

German trial in CD20+ PTLDs after SOT, children with no response to rituximab were allocated to a moderate six-cycle chemotherapy regimen (mCOMP: vincristine, prednisone, cyclophosphamide and methotrexate). Fifteen out of 49 children in the trial required mCOMP. Complete remission was achieved in ten cases; further, more intensive chemotherapy was required in four cases, and only one child died from chemotherapy toxicity. Complete remission was achieved also in 81% of the children who were treated with rituximab alone, and the overall survival was 86% (PedPTLD).

Another study was conducted in Bergamo to prospectively evaluate a risk-adapted approach to PTLD after diverse paediatric SOT. In this study children with severe PTLDs (monomorphic or multiorgan disease, or not responding to immunosuppression withdrawal) were stratified according to the disease risk. Only high-risk patients (based on histology, staging, general functioning and LDH level) were treated with a reduced-intensity polychemotherapy, with overall satisfactory outcomes (overall and disease-free survival: 82% and 75%, respectively) and no mortality due to antineoplastic toxicity [8].

The proposed risk-adapted treatment algorithm for paediatric PTLD shown in Fig. 34.2 is designed to minimise patients' overtreatment, considering that:

- Rituximab treatment alone is relatively safe but is associated with a high rate of disease progression [23].
- Mortality from toxic effects of cytoreductive agents is substantial, also adopting sequential approaches with rituximab and low-dose chemotherapy [23].
- Rituximab effectiveness could be underestimated by some study endpoints, due to delayed effect.

In brief, patients with PTLD can be managed with weaning of immunosuppression in case of polymorphic, localised disease. On the contrary, patients with a monomorphic histology, involvement of more than one organ or bone marrow, with a poor performance status or failing to respond to previous tapering of immunosuppression are considered affected by a more aggressive form and treated with rituximab. Furthermore, those with a disseminated disease (stage III or IV) and those with higher LDH levels are considered at higher risk and treated with an additional reduced chemotherapy protocol.

Other treatments have a marginal role. Antivirals have an impact only on the lytic phase of the viral cycle, which has a limited role in lymphoid hyperplasia. In any case there is no evidence that antivirals can improve the outcome of PTLD when used as adjunctive treatment [26].

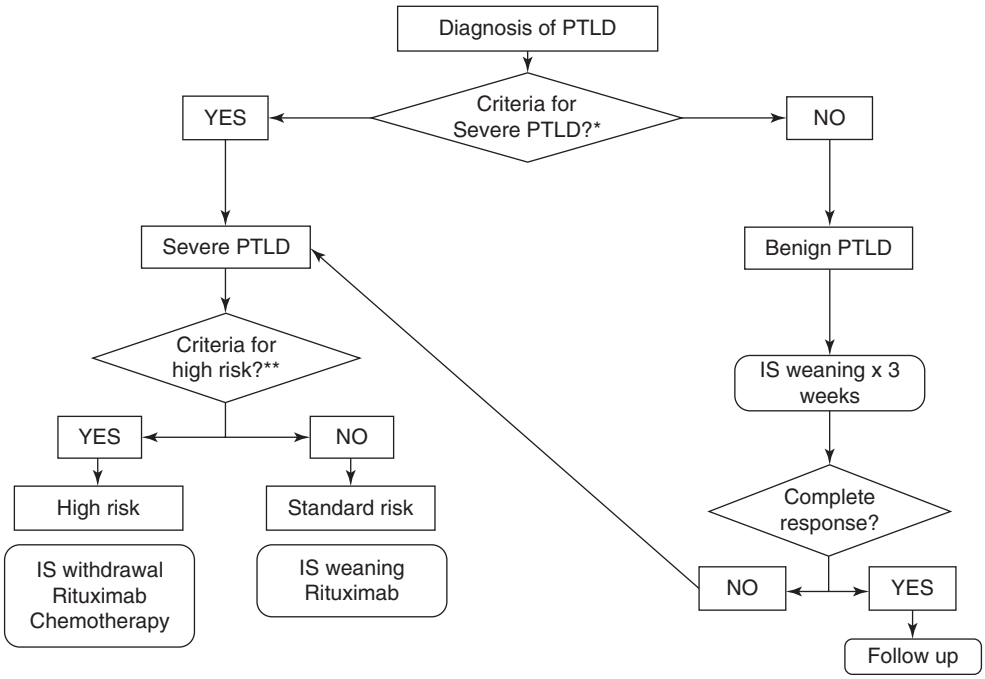

Fig. 34.2 Risk-adapted treatment algorithm for paediatric post-transplant lymphoproliferative disorder (PTLD) in solid organ transplantation. *Severe PTLD was diagnosed in the presence of at least one of the following criteria: (1) involvement of more than one organ, (2) involvement of the bone marrow, (3) organ dysfunction, (4) poor performance status, (5) monomorphic histology and (6) benign PTLD not responding to 3 weeks of immunosuppression withdrawal. **High-risk PTLD was diagnosed in the presence of at least two of the following criteria: (1) stage III or IV, (2) monomorphic histology, (3) poor performance status and (4) LDH ≥2 times the upper normal level for age (or ≥1000 IU/L). *IS* immunosuppression. In our centre, polychemotherapy includes blocks of fludarabine, cyclophosphamide, doxorubicin and rituximab (FCD-R) and reduced-intensity Berlin-Frankfurt-Münster (BFM) blocks for a maximum of six blocks

Since PTLD is a systemic disease, surgery and radiotherapy are useful only in selected cases, such as intestinal perforations (on intestinal localisations), or mass effect in central nervous system localisations.

Cell therapy strategies have been utilised to explore the competence of donor-derived and third-party EBV-specific T cells to restore the immunity against EBV, with promising but not definite results [27]. Another strategy is represented by the ex vivo production of EBV-specific T cells from autologous PBMC, obtained via the stimulation with lymphoblastoid cells as antigen-presenting cells [28]; however the limitation here is that these cells may not be rapidly available for a prompt clinical use.

34.3 CMV Infection and Disease

34.3.1 Clinical Pictures and Epidemiology

Cytomegalovirus (CMV) represents the most frequent opportunistic infection and a major threat in solid organ transplantation, especially in children. Because about 60% of adult donors have prior exposure to the virus, and most paediatric recipients receive an adult liver, the transplanted organ is often the source of infection.

In the absence of preventative measures (and similarly to EBV), the incidence of CMV infection (primary infection, reinfection or reactivation, defined as the detection of viral proteins or nucleic acid in any body fluid or tissue specimen), in this setting, depends mostly on the serological donor and recipient status. It is rare (1–2%) in case of both donor and recipient seronegativity; it occurs in 20–60% of the seropositive recipients as a reactivation, while almost all of the seronegative recipients receiving organs from seropositive donors get infected [29].

Another factor that has an impact on the risk of CMV infection and disease is the type of immunosuppression: the risk is greatest with lymphocyte-depleting agents and increased with the prolonged use of steroids and mycophenolic acid, when compared to standard tacrolimus monotherapy.

CMV infection in most cases has an asymptomatic course. However, about 20–30% of the infected patients develop a CMV disease, defined as the occurrence of consistent symptoms or tissue injury due to CMV [30, 31]. When overt, CMV disease most often causes a flu-like syndrome with fever, malaise, arthralgias and cytopenia. The virus can also cause direct injury to a wide range of target organs and tissues, due to its broad tropism. CMV is typically associated with colitis, retinitis, interstitial pneumonia and CNS disease with encephalitis, as well as other manifestations that are listed in Table 34.3. Importantly, CMV frequently causes graft hepatitis that resembles acute rejection. Beside these direct effects, it has been demonstrated that CMV replication is associated with "indirect" immunomodulatory effects ultimately leading to an increased risk of acute and chronic rejection, thrombotic events, opportunistic infections and PTLD [32, 33].

The combination of CMV infection and disease, in the absence of preventative measures, has been associated with a threefold higher risk of death or graft loss at 5 years post-LT [34–37].

34.3.2 Pathophysiology

CMV was historically perceived as a mild, slowly replicating virus capable of causing disease only in the presence of immune system impairment. However now it is regarded as a complex infection involving humoral and cellular, innate and adaptive immune responses.

CMV infection initially triggers innate immunity via the interaction of glycoprotein B- and Toll-like receptors and induces macrophage TLR4 and TLR5 ligand expression and TNF-alpha, IL-6 and IL-8 production. In parallel, NK cells stimulate the expression of IFN-gamma by effector

Table 34.3 Direct and indirect effects of cytomegalovirus in transplant recipients

Direct effects		Indirect effects	
CMV syndrome	Fever	Acute cellular rejection	
	Malaise	Chronic rejection	VBD syndrome
	Myelosuppression		Hepatitis
Tissue-invasive CMV disease	Colitis, enteritis		Fibrosis
	Hepatitis	Graft failure	
	Retinitis	Vascular thrombosis	
	Pneumonia/RDS	Opportunistic/secondary infections	Fungal
	Meningitis, encephalitis		Nocardia
	Carditis		Viruses (HHV6, EBV)
	MOF, death		PTLD

CMV cytomegalovirus, *RDS* respiratory distress syndrome, *MOF* multi-organ failure, *VBD* vanishing bile duct, *HHV6* human herpesvirus 6, *EBV* Epstein-Barr virus, *PTLD* post-transplant lymphoproliferative disorder. Modified from Marcelin JR et al.

cells and express Ig-like receptors which are relevant to viral control [38].

Turning to adaptive responses, the importance of humoral immunity is witnessed by the higher risk of developing CMV infection by seronegative recipients. However, pre-existing anti-CMV IgG may not protect from the strains introduced by the transplanted organ: among seropositive recipients, receiving a seropositive organ still increases the risk of CMV infection by threefold.

Finally, sustained control of CMV infection requires an adequate cellular response, as demonstrated by the crucial role of CMV-specific CD4 and CD8 cells in preventing CMV viremia and disease [39].

Data regarding the relationship between CMV infection and allograft tolerance are scarce and conflicting. Until recently, reports emphasised the correlation between CMV infection and disease and acute cellular rejection of the allograft, especially the liver [40], possibly as a consequence of the cross-reactivity of CMV-specific T-cell clones with allogeneic HLA molecules [41]. More recently, the link has been questioned, since most studies have not found an increased risk of acute rejection in CMV-infected patients [42–44] nor demonstrated a graft tolerogenic effect by the virus [45]. According to these latter findings, the hyporesponsiveness against the liver allograft would be accompanied and possibly caused by the relative shortage of donor-specific CD8 cells in liver allografts and by the higher Vdelta1/Vdelta2 γδ T-cell ratio, which is associated with operational tolerance [45].

34.3.3 Diagnosis

The diagnosis of CMV infection is based on the isolation of the virus or detection of viral proteins or nucleic acid in any body fluid or tissue specimen, regardless of symptoms. Nowadays, the best method is to use CMV-DNA detection via quantitative PCR. Both whole blood and plasma have proven suitable to determine and monitor the viral load and to provide prognostic information on the infection course [46]. However, decisional cut-off values in solid organ recipients have been better evaluated for whole blood samples [47–49]. Recently, the World Health Organization has released an international standard for CMV nucleic acid amplification technique (NIBSC 09/162) to homogenise the quantitative results worldwide.

Detection of the structural protein pp65 has lost importance, since it is technically more difficult and less precise than quantitative PCR and requires longer turnaround times. However, the equivalence between the two techniques is widely acknowledged.

CMV disease is defined as the evidence of infection in the presence of consistent symptoms, such as CMV syndrome (fever, malaise and myelosuppression) or proven CMV tissue-invasive disease (detection of CMV by immunohistochemical analysis and relevant histologic features on tissue biopsies, which are confirmed by in situ hybridisation or demonstration of CMV-DNA).

34.3.4 Treatment Algorithm

The goal of the anti-CMV management in the LT setting is to prevent overt disease and related complications. To achieve this result, two major strategies are currently employed: prophylaxis and preemptive therapy.

In the past, prophylaxis has been the most widely used approach and still remains the most used strategy by both the North American and the European paediatric transplant networks [50]. It consists of administering an antiviral agent (mainly ganciclovir or its orally absorbed prodrug valganciclovir) soon after LT to all, or only to high-risk, recipients regardless of the development of viremia, for a certain period of time. Undisputed advantages of this approach are that viral monitoring is not needed and infection does not develop soon after transplantation.

Preemptive therapy consists of administering the antiviral agents only in children with documented replication at an established viral load cut-off and continuing it till the obtainment of CMV-DNA clearance. The advantages of this approach are the reduced use of antiviral agents and the fact that a natural adaptive response is more rapidly achieved.

The pros and cons of each preventative strategy are showed in Table 34.4. However, any decision to choose either options should take into account the following issues:

– Overall outcomes do not differ between children who are managed with preemptive protocols and prophylaxis in terms of CMV disease incidence, graft-related

Table 34.4 Comparison between prophylaxis and preemptive therapy against cytomegalovirus

	Prophylaxis	Preemptive therapy
Early viremia/infection	Rare	Common
Late infection	Common	Rare
Prevention of CMV disease	Good efficacy	Good efficacy
Resistance	Uncommon	Uncommon (if weekly monitoring)
Feasibility	High	Needs CMV-DNA monitoring
Prevention of indirect effects	Unclear	Unclear
Costs	Drug costs	Monitoring costs
Drug exposure	High	Reduced
Graft survival	May improve	May improve

Modified from Kotton C, et al.

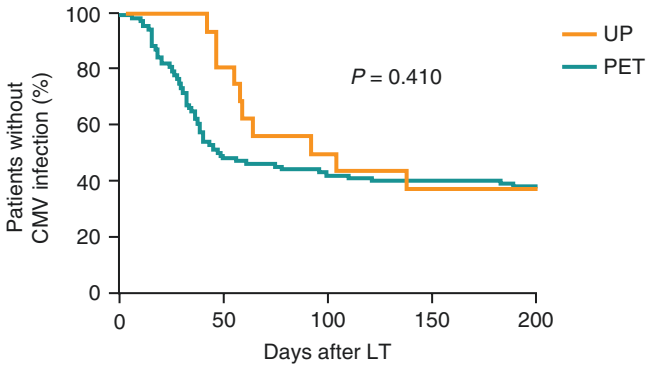

Fig. 34.3 Kaplan-Meier curve comparison showing the rate of cytomegalovirus (CMV) infection in patients managed with universal prophylaxis (UP) or preemptive therapy (PET) against cytomegalovirus. Although patients treated with UP get infected slightly later, by 100 days after transplantation, the rate of infection in the two groups is equal (see [44])

Table 34.5 Recommended preventative regimens against cytomegalovirus disease after LT in children

Serostatus	Risk level	Recommended	Alternative
D−/R−	Low	Monitoring of clinical symptoms	Preemptive therapy
D−/R+ D+/R+ D+/R−	Intermediate to high	2–4 weeks of GCV/VGCV with surveillance after prophylaxis[a]	3–4 months of VGCV Preemptive therapy

D donor, *R* recipient, *GCV* ganciclovir, *VGCV* valganciclovir
Modified from Kotton C, et al.
[a]Consider prolonged prophylaxis if T-cell-depleting agents

complications, graft loss and death, both in adults and children [44, 51, 52].
- Prophylaxis delays, but does not reduce, the overall infection rate by 200 days after LT (see Fig. 34.3, data of Bergamo Hospital) [44].
- Subclinical infection—which necessarily occurs with the preemptive approach—does not increase per se either the risk of acute cellular rejection nor that of other putative CMV indirect effects and has no effect on all causes of graft loss and death [44].
- Preemptive therapy is associated with less antiviral use, shorter length of hospitalisation and lower costs [44, 53].
- Virologic monitoring with acceptable turnaround time is a prerequisite for preemptive strategy.

Possible alternative preventative regimens recommended from the latest published guidelines are displayed in Table 34.5 [54]. In Bergamo, preemptive therapy is started at 100,000 CMV-DNA copies/mL (about 160,000 IU/mL) in whole blood samples (a cut-off established in a pilot study), regardless of the donor/recipient serostatus. This protocol has resulted in about 60% CMV infections and about 3% diseases, with no lethality associated to the virus. These results are similar to those recorded in other centres [31, 44, 52, 53]. Patients developing CMV disease are immediately treated with ganciclovir 5 mg/kg/dose every 12 h for at least 2 weeks and until resolution of symptoms and confirmed negative viremia. Other agents, such as foscarnet or cidofovir, are restricted to cases of suspected or confirmed resistance.

34.3.5 Management of CMV Antiviral Resistance

Drug resistance is defined as a viral genetic change that reduces the susceptibility of the virus to one or more antiviral drugs; it can present as a persistent or increasing viral load or the occurrence of symptoms despite adequate antiviral therapy [54]. The risk of harbouring resistance is present with both prophylaxis and preemptive protocols and is higher if the patient lacks pre-existing anti-CMV antibodies, in the case of highly immunosuppressed patients, or with inadequate antiviral drug delivery. Ganciclovir resistance has been reported to range from 5 to 12% in all solid organ transplant recipients [55–57], but it can be as high as 18 and 31% in lung and in intestinal transplant recipients, respectively [58–60]. The rate of viral resistance of other antiviral drugs is not well defined.

Patients who fail to achieve a substantial decrease in viral load after 2 weeks of treatment should be tested for resistance, especially if they have received a cumulative ganciclovir treatment longer than 6 weeks. Nowadays, the genotypic assay for viral resistance mutations in the UL97 kinase and UL54 DNA polymerase is the gold standard for the diagnosis. Interpretation of genetic testing should be conducted by an expert virologist. However, it is important to understand that the seven most common mutations in UL97 account for 80% of resistance pattern and that mutations in this gene do not confer cross-resistance to other drugs. On the other hand, mutations in UL54 occur in more conserved domains, and cross-resistance to foscarnet, cidofovir or both is likely.

For some low-grade resistance mutations (those that increase by two- to fivefold the drug concentration reducing viral growth by 50% [EC50]), doubling the ganciclovir dose may be sufficient and reduces the immunosuppression, if feasible. In other cases, a drug switch guided by the resistance test is recommended.

Alternative antiviral drugs are mainly foscarnet and cidofovir. Many other treatments are in development. Maribavir has succeeded as a salvage therapy in extensively resistant cases [54]. Letermovir is a potent UL56 terminase inhibitor and is being tested in prophylaxis regimens and as a rescue treatment in cases of resistant strains [61].

References

1. Shepherd RW, Turmelle Y, Nadler M, Lowell JA, Narkewicz MR, McDiarmid SV, et al. Risk factors for rejection and infection in pediatric liver transplantation. Am J Transplant. 2008;8:396–403. https://doi.org/10.1111/j.1600-6143.2007.02068.x.
2. Green M. Viral infections and pediatric liver transplantation. Pediatr Transplant. 2002;6:20–4.
3. Cox KL, Lawrence-Miyasaki LS, Garcia-Kennedy R, Lennette ET, Martinez OM, Krams SM, et al. An increased incidence of Epstein-Barr virus infection and lymphoproliferative disorder in young children on FK506 after liver transplantation. Transplantation. 1995;59:524–9.
4. Narkewicz MR, Green M, Dunn S, Millis M, McDiarmid S, Mazariegos G, et al. Decreasing incidence of symptomatic Epstein-Barr virus disease and posttransplant lymphoproliferative disorder in pediatric liver transplant recipients: report of the studies of pediatric liver transplantation experience. Liver Transpl. 2013;19:730–40. https://doi.org/10.1002/lt.23659.
5. Sokal EM, Antunes H, Beguin C, Bodeus M, Wallemacq P, de Ville de Goyet J, et al. Early signs and risk factors for the increased incidence of Epstein-Barr virus-related posttransplant lymphoproliferative diseases in pediatric liver transplant recipients treated with tacrolimus. Transplantation. 1997;64:1438–42.
6. Cacciarelli TV, Reyes J, Jaffe R, Mazariegos GV, Jain A, Fung JJ, et al. Primary tacrolimus (FK506) therapy and the long-term risk of post-transplant lymphoproliferative disease in pediatric liver transplant recipients. Pediatr Transplant. 2001;5:359–64.
7. Younes BS, McDiarmid SV, Martin MG, Vargas JH, Goss JA, Busuttil RW, et al. The effect of immunosuppression on post-transplant lymphoproliferative disease in pediatric liver transplant patients. Transplantation. 2000;70:94–9.
8. Giraldi E, Provenzi M, Conter V, Colledan M, Bolognini S, Foglia C, et al. Risk-adapted treatment for severe B-lineage posttransplant lymphoproliferative disease after solid organ transplantation in children. Transplantation. 2016;100:437–45. https://doi.org/10.1097/TP.0000000000000845.
9. Cao S, Cox KL, Berquist W, Hayashi M, Concepcion W, Hammes GB, et al. Long-term outcomes in pediatric liver recipients: comparison between cyclosporin A and tacrolimus. Pediatr Transplant. 1999;3:22–6.
10. Wistinghausen B, Gross TG, Bollard C. Post-transplant lymphoproliferative disease in pediatric solid organ transplant recipients. Pediatr Hematol Oncol. 2013;30:520–31. https://doi.org/10.3109/08880018.2013.798844.
11. Llaurador G, McLaughlin L, Wistinghausen B. Management of post-transplant lymphoproliferative disorders. Curr Opin Pediatr. 2017;29:34–40. https://doi.org/10.1097/MOP.0000000000000445.
12. D'Antiga L, Del Rizzo M, Mengoli C, Cillo U, Guariso G, Zancan L. Sustained Epstein-Barr virus detection in paediatric liver transplantation. Insights into the occurrence of late PTLD. Liver Transpl. 2007;13:343–8. https://doi.org/10.1002/lt.20958.
13. Münz C. Epstein Barr virus - a tumor virus that needs cytotoxic lymphocytes to persist asymptomatically. Curr Opin Virol. 2016;20:34–9. https://doi.org/10.1016/j.coviro.2016.08.010.
14. Miller G, El-Guindy A, Countryman J, Ye J, Gradoville L. Lytic cycle switches of oncogenic human gammaherpesviruses. Adv Cancer Res. 2007;97:81–109. https://doi.org/10.1016/S0065-230X(06)97004-3.
15. Whitehurst CB, Li G, Montgomery SA, Montgomery ND, Su L, Pagano JS. Knockout of Epstein-Barr virus BPLF1 retards B-cell transformation and lymphoma formation in humanized mice. MBio. 2015;6:e01574–15. https://doi.org/10.1128/mBio.01574-15.
16. Morscio J, Tousseyn T. Recent insights in the pathogenesis of post-transplantation lymphoproliferative disorders. World J Transplant. 2016;6:505–16. https://doi.org/10.5500/wjt.v6.i3.505.
17. Savoie A, Perpête C, Carpentier L, Joncas J, Alfieri C. Direct correlation between the load of Epstein-Barr virus-infected lymphocytes in the peripheral blood of pediatric transplant patients and risk of lymphoproliferative disease. Blood. 1994;83:2715–22.
18. Gridelli B, Spada M, Riva S, Colledan M, Segalin A, Lucianetti A, et al. Circulating Epstein-Barr virus DNA to monitor lymphoproliferative disease following pediatric liver transplantation. Transpl Int. 2000;13(Suppl 1):S399–401.
19. Macedo C, Zeevi A, Bentlejewski C, Popescu I, Green M, Rowe D, et al. The impact of EBV load on T-cell immunity in pediatric thoracic transplant recipients. Transplantation. 2009;88:123–8. https://doi.org/10.1097/TP.0b013e3181aacdd7.
20. Lee TC, Goss JA, Rooney CM, Heslop HE, Barshes NR, Caldwell YM, et al. Quantification of a low cellular immune response to aid in identification of pediatric liver transplant recipients at high-risk for EBV infection. Clin Transpl. 2006;20:689–94. https://doi.org/10.1111/j.1399-0012.2006.00537.x.
21. Reshef R, Vardhanabhuti S, Luskin MR, Heitjan DF, Hadjiliadis D, Goral S, et al. Reduction of immunosuppression as initial therapy for posttransplantation lymphoproliferative disorder(★). Am J Transplant. 2011;11:336–47. https://doi.org/10.1111/j.1600-6143.2010.03387.x.
22. Styczynski J, Gil L, Tridello G, Ljungman P, Donnelly JP, van der Velden W, et al. Response to rituximab-based therapy and risk factor analysis in Epstein Barr Virus-related lymphoproliferative disorder after hematopoietic stem cell transplant in children and adults: a study from the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. Clin Infect Dis. 2013;57:794–802. https://doi.org/10.1093/cid/cit391.
23. Trappe R, Oertel S, Leblond V, Mollee P, Sender M, Reinke P, et al. Sequential treatment with rituximab followed by CHOP chemotherapy in adult B-cell post-transplant lymphoproliferative disorder (PTLD): the prospective international multicentre phase 2 PTLD-1 trial. Lancet Oncol. 2012;13:196–206. https://doi.org/10.1016/S1470-2045(11)70300-X.
24. Dotti G, Fiocchi R, Motta T, Mammana C, Gotti E, Riva S, et al. Lymphomas occurring late after solid-organ transplantation: influence of treatment on the clinical outcome. Transplantation. 2002;74:1095–102. https://doi.org/10.1097/01.TP.0000030637.87507.3C.
25. Elstrom RL, Andreadis C, Aqui NA, Ahya VN, Bloom RD, Brozena SC, et al. Treatment of PTLD with rituximab or chemotherapy. Am J Transplant. 2006;6:569–76. https://doi.org/10.1111/j.1600-6143.2005.01211.x.
26. Østensen AB, Sanengen T, Holter E, Line P-D, Almaas R. No effect of treatment with intravenous ganciclovir on Epstein-Barr virus viremia demonstrated after pediatric liver transplantation. Pediatr Transplant. 2017;21:e13010. https://doi.org/10.1111/petr.13010.
27. Doubrovina E, Oflaz-Sozmen B, Prockop SE, Kernan NA, Abramson S, Teruya-Feldstein J, et al. Adoptive immunotherapy with unselected or EBV-specific T cells for biopsy-proven EBV+ lymphomas after allogeneic hematopoietic cell transplantation. Blood. 2012;119:2644–56. https://doi.org/10.1182/blood-2011-08-371971.
28. Gottschalk S, Rooney CM. Adoptive T-Cell Immunotherapy. Curr Top Microbiol Immunol. 2015;391:427–54. https://doi.org/10.1007/978-3-319-22834-1_15.
29. Razonable RR, Humar A. AST Infectious Diseases Community of Practice. Cytomegalovirus in solid organ transplantation. Am J Transplant. 2013;13(Suppl 4):93–106. https://doi.org/10.1111/ajt.12103.
30. Winston DJ, Emmanouilides C, Busuttil RW. Infections in liver transplant recipients. Clin Infect Dis. 1995;21:1077–89; quiz 1090–1.
31. Bruminhent J, Razonable RR. Management of cytomegalovirus infection and disease in liver transplant recipients. World J Hepatol. 2014;6:370–83. https://doi.org/10.4254/wjh.v6.i6.370.

32. Marcelin JR, Beam E, Razonable RR. Cytomegalovirus infection in liver transplant recipients: updates on clinical management. World J Gastroenterol. 2014;20:10658–67. https://doi.org/10.3748/wjg.v20.i31.10658.
33. Freeman HJ. Long-term natural history of Crohn's disease. World J Gastroenterol. 2009;15:1315–8.
34. Bosch W, Heckman MG, Diehl NN, Shalev JA, Pungpapong S, Hellinger WC. Association of cytomegalovirus infection and disease with death and graft loss after liver transplant in high-risk recipients. Am J Transplant. 2011;11:2181–9. https://doi.org/10.1111/j.1600-6143.2011.03618.x.
35. Razonable RR. Cytomegalovirus infection after liver transplantation: current concepts and challenges. World J Gastroenterol. 2008;14:4849–60. https://doi.org/10.3748/wjg.14.4849.
36. Stratta RJ, Shaefer MS, Markin RS, Wood RP, Kennedy EM, Langnas AN, et al. Clinical patterns of cytomegalovirus disease after liver transplantation. Arch Surg. 1989;124:1443–9; discussion 1449–50.
37. Burak KW, Kremers WK, Batts KP, Wiesner RH, Rosen CB, Razonable RR, et al. Impact of cytomegalovirus infection, year of transplantation, and donor age on outcomes after liver transplantation for hepatitis C. Liver Transpl. 2002;8:362–9. https://doi.org/10.1053/jlts.2002.32282.
38. Carbone J. The immunology of posttransplant CMV infection: potential effect of CMV immunoglobulins on distinct components of the immune response to CMV. Transplantation. 2016;100(Suppl 3):S11–8. https://doi.org/10.1097/TP.0000000000001095.
39. Manuel O, Husain S, Kumar D, Zayas C, Mawhorter S, Levi ME, et al. Assessment of cytomegalovirus-specific cell-mediated immunity for the prediction of cytomegalovirus disease in high-risk solid-organ transplant recipients: a multicenter cohort study. Clin Infect Dis. 2013;56:817–24. https://doi.org/10.1093/cid/cis993.
40. Gupta P, Hart J, Cronin D, Kelly S, Millis JM, Brady L. Risk factors for chronic rejection after pediatric liver transplantation. Transplantation. 2001;72:1098–102.
41. Amir AL, D'Orsogna LJA, Roelen DL, van Loenen MM, Hagedoorn RS, de Boer R, et al. Allo-HLA reactivity of virus-specific memory T cells is common. Blood. 2010;115:3146–57. https://doi.org/10.1182/blood-2009-07-234906.
42. Sun H-Y, Cacciarelli TV, Wagener MM, Singh N. Preemptive therapy for cytomegalovirus based on real-time measurement of viral load in liver transplant recipients. Transpl Immunol. 2010;23:166–9. https://doi.org/10.1016/j.trim.2010.06.013.
43. Indolfi G, Heaton N, Smith M, Mieli-Vergani G, Zuckerman M. Effect of early EBV and/or CMV viremia on graft function and acute cellular rejection in pediatric liver transplantation. Clin Transpl. 2012;26:E55–61. https://doi.org/10.1111/j.1399-0012.2011.01535.x.
44. Nicastro E, Giovannozzi S, Stroppa P, Casotti V, Callegaro AP, Tebaldi A, et al. Effectiveness of preemptive therapy for cytomegalovirus disease in pediatric liver transplantation. Transplantation. 2017;101:804–10. https://doi.org/10.1097/TP.0000000000001531.
45. Shi X-L, de Mare-Bredemeijer ELD, Tapirdamaz Ö, Hansen BE, van Gent R, van Campenhout MJH, et al. CMV primary infection is associated with donor-specific T cell hyporesponsiveness and fewer late acute rejections after liver transplantation. Am J Transplant. 2015;15:2431–42. https://doi.org/10.1111/ajt.13288.
46. Boaretti M, Sorrentino A, Zantedeschi C, Forni A, Boschiero L, Fontana R. Quantification of cytomegalovirus DNA by a fully automated real-time PCR for early diagnosis and monitoring of active viral infection in solid organ transplant recipients. J Clin Virol. 2013;56:124–8. https://doi.org/10.1016/j.jcv.2012.10.015.
47. Allice T, Cerutti F, Pittaluga F, Varetto S, Franchello A, Salizzoni M, et al. Evaluation of a novel real-time PCR system for cytomegalovirus DNA quantitation on whole blood and correlation with pp65-antigen test in guiding pre-emptive antiviral treatment. J Virol Methods. 2008;148:9–16. https://doi.org/10.1016/j.jviromet.2007.10.006.
48. Gerna G, Lilleri D, Furione M, Baldanti F. Management of human cytomegalovirus infection in transplantation: validation of virologic cut-offs for preemptive therapy and immunological cut-offs for protection. New Microbiol. 2011;34:229–54.
49. Lilleri D, Lazzarotto T, Ghisetti V, Ravanini P, Capobianchi MR, Baldanti F, et al. Multicenter quality control study for human cytomegalovirus DNAemia quantification. New Microbiol. 2009;32:245–53.
50. Danziger-Isakov L, Bucavalas J. Current prevention strategies against cytomegalovirus in the studies in pediatric liver transplantation (SPLIT) centers. Am J Transplant. 2014;14:1908–11. https://doi.org/10.1111/ajt.12755.
51. Mumtaz K, Faisal N, Husain S, Morillo A, Renner EL, Shah PS. Universal prophylaxis or preemptive strategy for cytomegalovirus disease after liver transplantation: a systematic review and meta-analysis. Am J Transplant. 2015;15:472–81. https://doi.org/10.1111/ajt.13044.
52. Saitoh A, Sakamoto S, Fukuda A, Shigeta T, Kakiuchi T, Kamiyama S, et al. A universal preemptive therapy for cytomegalovirus infections in children after live-donor liver transplantation. Transplantation. 2011;92:930–5. https://doi.org/10.1097/TP.0b013e31822d873d.
53. Gerna G, Lilleri D, Callegaro A, Goglio A, Cortese S, Stroppa P, et al. Prophylaxis followed by preemptive therapy versus preemptive therapy for prevention of human cytomegalovirus disease in pediatric patients undergoing liver transplantation. Transplantation. 2008;86:163–6. https://doi.org/10.1097/TP.0b013e31817889e4.
54. Kotton CN, Kumar D, Caliendo AM, Huprikar S, Chou S, Danziger-Isakov L, et al. The third international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. Transplantation. 2018;102:900–31. https://doi.org/10.1097/TP.0000000000002191.
55. Hantz S, Garnier-Geoffroy F, Mazeron M-C, Garrigue I, Merville P, Mengelle C, et al. Drug-resistant cytomegalovirus in transplant recipients: a French cohort study. J Antimicrob Chemother. 2010;65:2628–40. https://doi.org/10.1093/jac/dkq368.
56. Myhre H-A, Haug Dorenberg D, Kristiansen KI, Rollag H, Leivestad T, Asberg A, et al. Incidence and outcomes of ganciclovir-resistant cytomegalovirus infections in 1244 kidney transplant recipients. Transplantation. 2011;92:217–23. https://doi.org/10.1097/TP.0b013e31821fad25.
57. Young PG, Rubin J, Angarone M, Flaherty J, Penugonda S, Stosor V, et al. Ganciclovir-resistant cytomegalovirus infection in solid organ transplant recipients: a single-center retrospective cohort study. Transpl Infect Dis. 2016;18:390–5. https://doi.org/10.1111/tid.12537.
58. Boivin G, Goyette N, Rollag H, Jardine AG, Pescovitz MD, Asberg A, et al. Cytomegalovirus resistance in solid organ transplant recipients treated with intravenous ganciclovir or oral valganciclovir. Antivir Ther (Lond). 2009;14:697–704.
59. Lurain NS, Bhorade SM, Pursell KJ, Avery RK, Yeldandi VV, Isada CM, et al. Analysis and characterization of antiviral drug-resistant cytomegalovirus isolates from solid organ transplant recipients. J Infect Dis. 2002;186:760–8. https://doi.org/10.1086/342844.
60. Ambrose T, Sharkey LM, Louis-Auguste J, Rutter CS, Duncan S, English S, et al. Cytomegalovirus infection and rates of antiviral resistance following intestinal and multivisceral transplantation. Transplant Proc. 2016;48:492–6. https://doi.org/10.1016/j.transproceed.2015.09.070.
61. Chemaly RF, Ullmann AJ, Stoelben S, Richard MP, Bornhäuser M, Groth C, et al. Letermovir for cytomegalovirus prophylaxis in hematopoietic-cell transplantation. N Engl J Med. 2014;370:1781–9. https://doi.org/10.1056/NEJMoa1309533.