Clinical Features and Diagnostic Considerations

8

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Introduction

The Epstein-Barr virus (EBV) is associated with the majority of cases of post-transplant lymphoproliferative disorder (PTLD). This condition encompasses a spectrum of clinical entities in the post-transplant period. These syndromes range from the manifestations of non-destructive lesions, including infectious mononucleosis-like pathologies, to true malignancies [1]. While these manifestations of PTLD are often conveniently classified into discreet entities, in reality they often represent a spectrum of illnesses where more benign entities may be followed by more serious syndromes. The heterogeneous nature of PTLD makes generalization problematic. This notwithstanding, one can recognize two primary modes of presentation of PTLD in the solid organ transplant recipient, namely, early-onset PTLD and later-onset PTLD. Although the time demarcation between these entities is somewhat arbitrary, the former occurs within the first 1–2 years, while the latter occurs after the first 1–2 years [2].

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Severe Infectious Mononucleosis, Clinical Categories, and Sites of PTLD

Severe Infectious Mononucleosis Infectious mononucleosis is the prototype of primary EBV infection [3, 4]. The clinical spectrum of infection ranges from asymptomatic infection to severe, sometimes fatal disease in immunocompromised patients. Infectious mononucleosis is typically characterized by fever, exudative pharyngitis, lymphadenopathy, hepatosplenomegaly, and atypical lymphocytosis. In symptomatic individuals, adenotonsillar disease is often a prominent feature (Fig. 8.1). The features of severe infectious mononucleosis may be seen in some cases of acutely symptomatic PTLD. In complicated cases or the more severe cases in the immunocompromised host, patients may develop hepatitis, upper airway obstruction due to enlarged adenotonsillar tissue, pneumonitis, encephalitis, aseptic meningitis, splenic rupture, decreased blood cellular elements, disseminated intravascular coagulation and hemophagocytic syndrome, bacterial superinfection, and renal, cardiac, and other complications [3, 4]. In the transplant setting, certain features (e.g., hepatitis) may be accentuated or represent diagnostic dilemmas as this relates to the role that the virus is having versus non-EBV-related complications of transplantation.

PTLD Presenting Early After Organ Transplantation PTLD presenting within the first 1–2 years after transplantation may be characterized by marked constitutional symptoms and rapid enlargement of lymphoreticular tissue. The vast majority of these lesions during this time are EBV-positive. Although less commonly seen in recent years, this entity is characterized by rapidly progressive disease of a disseminated nature and a systemic sepsis-like syndrome as a result of a cytokine storm. The clinical picture includes some features that are consistent with severe EBV disease [5], as outlined above (e.g., hemophagocytosis and disseminated intravascular coagulation). In some patients, the diagnosis of PTLD is unfortunately

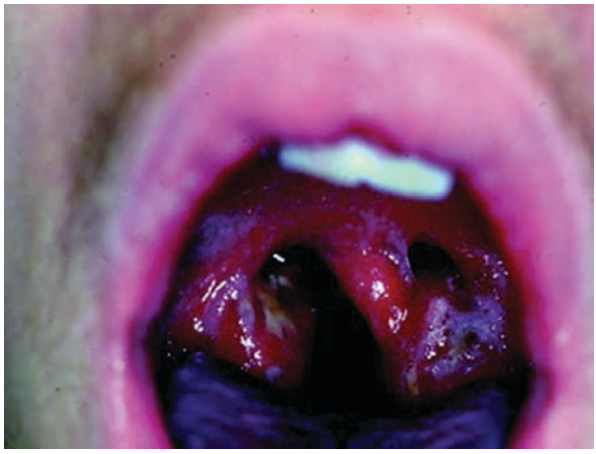

Fig. 8.1 Exudative tonsillopharyngitis in infectious mononucleosis. (Footnote: Reproduced with permission, Slide Library, Hospital for Sick Children, Toronto)

made at autopsy due to difficulty in diagnosis [6, 7]. Mass lesions may not be present; pyrexia is present and the disease may be extranodal. It can be difficult to separate this entity from patients who have overwhelming sepsis and multiorgan failure. The above notwithstanding, some cases of early PTLD may present in less aggressive forms with nodal involvement and less constitutional symptomatology. In the adult patient, this presentation is the most frequent presentation of the early-onset PTLD.

PTLD Presenting Late After Organ Transplantation PTLD that presents after the first 1–2 years after transplantation is likely to be more anatomically defined, has few systemic symptoms, and is less rapidly progressive. This form of PTLD is now the form that is frequently seen in most centers, as the early-onset, rapidly progressive form is less frequently seen in recent years [8, 9]. Proportionately more cases of EBV-negative PTLD occur in the late-onset category in contrast to the early-onset category. One possible explanation is that in recent years, the enhanced surveillance for EBV after transplantation has enabled the early recognition of upregulation of EBV activity prior to the development of PTLD, allowing for early intervention, including reduction in immunosuppression.

PTLD Occurring After Hematopoietic Stem Cell Transplantation This is addressed elsewhere in this book and will only be briefly mentioned here for context and contrast. In the HSCT patients, PTLD usually affects recipients of allogeneic grafts. Among affected patients, very few cases of PTLD occur after the first year in the absence of chronic graft versus host disease. This is due to the fact that immune restoration occurs as engraftment takes place with advancing time after HSCT. This is in contrast to solid organ transplant recipients who require varying degrees of ongoing immunosuppression to prevent organ rejection. The occurrence of PTLD at a relatively early stage after HSCT poses a challenge with a tendency for fulminant multi-system disease in some patients. While HSCT patients may experience the full spectrum of PTLD seen in solid organ transplantations, it occurs significantly less frequently after hematopoietic stem cell transplantation (HSCT) compared with solid organ transplantation. Among HSCT recipients, PTLD lesions are usually of donor origin in contrast to recipient origin in the majority of solid organ transplant recipients [10–12].

Sites of PTLD Lesions The dominant presenting signs and symptoms of PTLD are related to the organs affected and the sites of PTLD lesions. In contrast to lymphomas in immunocompetent patients, PTLD is associated with a very high incidence of extranodal involvement, with published rates of 60–90%. Virtually no site is exempt from PTLD involvement, and a high index of suspicion is required when assessing lesions in any location in the body of patients after transplantation. In this regard, PTLD has been documented at the following sites: bone, bone marrow, small bowel, large bowel, stomach, central nervous system, diaphragm, kidneys, liver, lung, lymph nodes, orbits, ovary, paraspinal tissues, salivary glands, paranasal sinuses, skin, soft palate, spleen, stomach, testes, tonsils, and uterus. In addition,

EBV-positive (+) mucocutaneous ulcers involving the oropharyngeal mucosa, skin, or gastrointestinal tract may occur and have been added to the WHO classification system [1, 13].

The vast majority of cases involve the organs of the reticuloendothelial systems and the transplanted organs. With respect to the transplanted organs, the heart is the only organ that is not usually the primary site of PTLD. Data from a recent review of PTLD cases in children over a 15-year period at The Hospital for Sick Children in Toronto revealed that the sites most frequently affected at the diagnosis of PTLD were tonsillar/adenoidal (34%), gastrointestinal (GI) tract (32%), lymph node (LN) 11%), and multisite (11%) [14]. Among adult patients, Caillard et al. described a temporal sequence of sites of PTLD among renal transplant recipients, with disease localized to the graft occurring within the first 2 years, primary CNS lymphoma (PCNSL) occurring between years 2 and 7, and gastrointestinal disease occurring between years 6 and 10 and being the predominant site of late disease [15], the latter supporting the observation of the relatively high frequency of involvement of the GI tract in cases of PTLD [16–18].

With respect to GI tract disease, the nature of organ involvement may include isolated solitary or multisite lesions or disease that is part of a more disseminated process. Easily resectable intestinal lesions that are solitary are associated with better outcomes compared with disease that is either multisite or part of a more generalized PTLD process. Patients with GI PTLD may present with a variety of gastrointestinal manifestations, including vomiting, diarrhea, evidence of protein-losing enteropathy, intussusception, bleeding, and in some cases evidence of bowel perforation. The latter is also a known complication during the treatment phase of intestinal PTLD during which necrosis of transmural lesions can occur.

Patients with head and neck PTLD disease may present with a spectrum of findings including asymptomatic adenotonsillar hypertrophy, tonsillitis, palatal ulcerative lesions, cervical lymphadenopathy, and disease of the paranasal sinuses [19–24]. The latter has been documented to be one of the manifestations of PTLD in patients who have undergone lung transplantation [24]. Among these findings, enlarged adenoids and tonsils represent the most frequent presentation of head and neck PTLD (Fig. 8.2). In one series, adenotonsillar biopsies yielded PTLD in approximately 40% of children who were referred to the otolaryngology service for assessment to rule out PTLD following initial screening by clinicians [19].

Pulmonary involvement is most frequently seen in heart and lung transplant patients. In most cases it is characterized by solitary or multiple pulmonary nodules or an infiltrative process [7, 15, 25, 26]. In addition, there may be pulmonary dysfunction in the lung allograft. In the latter situation, clearly discernible lesions might not be apparent in the setting of diffuse consolidation on chest X-rays.

Liver involvement usually occurs in liver transplant recipients where there may be evidence of diffuse hepatitis or nodular disease. Non-liver transplant recipients may also have liver involvement as a component of multi-system disease.

Among renal transplant recipients, PTLD may involve the allograft or distant sites. This influences the nature of the presenting signs and symptoms. When PTLD

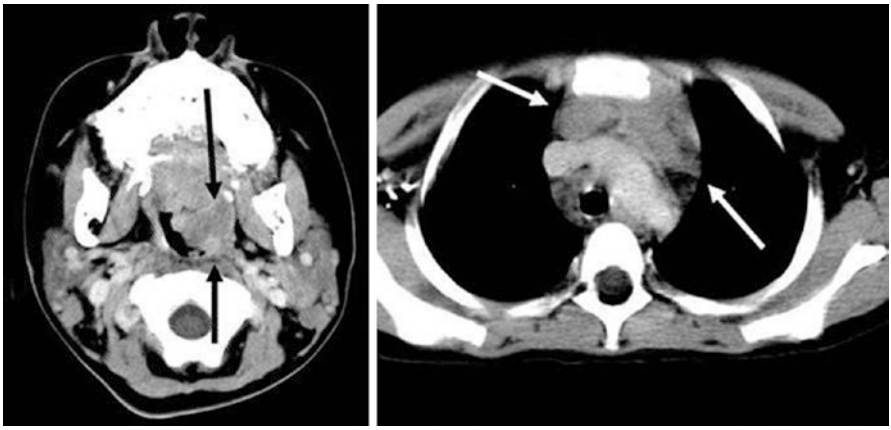

Fig. 8.2 Older child after liver transplantation. CT reveals left tonsillar nodal mass and mediastinal adenopathy. (Courtesy of Dr. David Manson, Hospital for Sick Children, Toronto)

affects the renal graft, a significant proportion of patients may present with renal dysfunction [15]. However, when alterations in renal function occur presumably due to PTLD affecting the kidneys, other cause of renal dysfunction after transplant should be considered in the differential diagnosis. These conditions include rejection and BK polyoma virus nephropathy.

Patients may also present with skin nodules. These should be differentiated from non-PTLD malignancies, including donor-derived malignancies in adult patients. Rarely, EBV-associated smooth muscle tumors have been described [27].

Central nervous system (CNS) disease is usually seen in the setting of extensive multi-system disease. However, solitary CNS disease may occur, which is an important consideration in the diagnostic evaluation of transplant patients with sustained elevations of EBV loads. In this regard, patients with CNS lesions might not have symptoms or signs referable to the CNS during the early stages of disease. The time to primary CNS PTLD may be less than 2 years and exceed 10 years post-transplant [15, 28, 29]. When symptomatic, patients may present with evidence of intracranial pathology with headaches, seizures, and focal neurologic deficits. Generally, patients presenting with CNS PTLD tend to have poorer prognoses [8, 15, 25].

As indicated above, several other sites may be affected by PTLD. Their clinical importance may relate to the fact that their involvement may be indicative of disseminated disease and/or may be suggestive of poorer outcomes. For example, as is the case with CNS PTLD, bone marrow involvement is regarded as a poor prognostic indicator.

Histopathologic Correlates

The histopathologic examination of suspected PTLD lesions is crucial for the diagnosis of PTLD [1, 30, 31]. A detailed description is provided in Chap. 2; PTLD lesions presenting early after transplantation are generally EBV-associated.

Non-destructive lesions of the plasmacytic and infectious mononucleosis types tend to occur at a younger age than other forms of PTLD and are thus more likely to be seen in children than adults [1]. These lesions tend to occur in primary EBV where infection occurs in the setting of no previous exposure to the virus.

The histology of PTLD lesions presenting late after transplantation is highly variable. In children and adults experiencing late-onset primary EBV infection, "non-destructive PTLD" and/or other forms of PTLD may still be observed. With increasing time from transplantation, a greater proportion of lesions are monomorphic, and many are EBV-negative, notably in adults. These lesions may resemble non-Hodgkin lymphomas, Hodgkin lymphoma, or malignancies with plasma cell predominance. Their clinical behaviors are variable and may be different from the histologically equivalent lesions in non-transplant recipients. Monomorphic lesions are clonal proliferations, and genetic abnormalities and structural chromosomal changes are much more prevalent than in polymorphic lesions.

Diagnostic Evaluation

Early diagnosis of PTLD is essential in order to maximize favorable outcomes. The initial diagnostic evaluation of patients with suspected PTLD is influenced by the appropriate historical information, as this relates to symptoms as well as background patient information and the physical examination findings. The diagnostic workup is guided by the presenting symptoms and signs as outlined above and in Table 8.1, taking into account the differential diagnosis. Therefore, clinicians need to be aware of the conditions that must be differentiated from PTLD in order that these alternative diagnoses are not missed and are managed appropriately.

Table 8.1 Presenting symptoms and signs in patients with lymphoproliferative disorder

Symptoms/complaints	Signs
Swollen lymph glands	Lymphadenopathy
Weight loss	Hepatosplenomegaly
Fever or night sweats	Subcutaneous nodules
Sore throat	Tonsillar enlargement
Malaise and lethargy	Tonsillar inflammation
Chronic sinus congestion and discomfort	Signs of bowel perforation
Anorexia, nausea, and vomiting	Focal neurologic signs
Abdominal pain	
Gastrointestinal bleeding	
Symptoms of bowel perforation	
Cough and shortness of breath	
Headache	
Focal neurologic deficits	

Background Information on Patients

Clinical information that should be recorded includes the patient's age, the underlying disease resulting in transplantation, the date(s) and type(s) of transplant received, and the date of onset of symptoms. It is also necessary to obtain other information that will assist in determining the risk of PTLD or guide the subsequent management of the patient [32, 33]. This is covered in detail in Chap. 10. The donor and recipient EBV serostatus is important given the fact that the primary risk factor for PTLD in the SOT patient is primary EBV infection [32, 33]. Pediatric patients are more likely to have primary EBV infection after transplantation, due to the fact the majority are EBV-seronegative at transplantation compared with their adult counterparts. Additional data include the types of organ transplanted and the dose and types of immunosuppression used. In this regard, the risk of PTLD depends on the types of organ transplanted. Patients who have received specific anti-T cell therapies may be at an increased risk of PTLD [33], although in recent years, clinical experiences are less convincing. The types and doses of antiviral agents used and the CMV donor and recipient serostatus are relevant, given the possibility that CMV infection/disease may be a risk factor 32–33],

Initial Clinical Examination

In keeping with regular clinical practice, a thorough physical examination is required to detect the manifestations of PTLD, which may be quite nonspecific (Table 8.1). The general physical examination might elicit evidence of pallor or signs referable to the site(s) of organs affected by PTLD. Given the predilection for the reticuloendothelial system to be involved, the clinical examination should include a meticulous assessment for lymphadenopathy. In selected cases, clinicians may choose to supplement clinical examinations with chest radiographs and abdominal ultrasounds as they screen for lymphadenopathy. The clinical examination should include periodic assessments by an otolaryngologist in high-risk cases, given the frequency with which the adenotonsillar tissues are involved, notably in the setting of primacy EBV infection.

Diagnostic and Screening Tests

The diagnostic tests that are performed for PTLD can be group into four main categories (Table 8.2). These are (1) general tests; (2) non-EBV-specific tests; (3) EBV-specific tests; and (4) histopathology. Given the importance of early diagnosis, the development of screening tests has been the subject of research for many years. Such screening is aimed at detecting subclinical PTLD or more overt PTLD in its earliest stages. There are data to suggest that in some patients, a definite subclinical phase of PTLD exists [34]. This is based on examination of liver biopsy samples

Table 8.2 Diagnostic evaluation of patients with symptoms or signs consistent with PTLD

General tests	Selective diagnostic tests	EBV-specific
CBC, WBC differential	Evaluation for specific infectious agents based on clinical presentation	EBV serologies (anti-EA, EBNA, and VCA)
Liver function tests	Lumbar puncture	EBV viral load in peripheral blood/blood compartments
Renal function tests	Bone scan	EBV status of lesions (PCR, in situ hybridization)
Serum electrolytes, calcium	Bone marrow biopsy	Excision or core needle biopsy of lesions for histopathology
Lactate dehydrogenase	Brain CT/MRI	
Uric acid	Gastrointestinal endoscopy	
Serum immunoglobulins	PET scan	
Stools for occult blood		
Chest radiographs		
CT scan of chest/abdomen/pelvis		

obtained prior to the diagnosis of PTLD. Examination of such samples have indicated the presence of EBV by PCR or EBER staining in 70% of patients who went on to develop PTLD compared with 10% of those who did not develop PTLD [34]. In addition, the histopathological examination of enlarged adenoidal tissue may indicate evidence of occult PTLD in asymptomatic individuals. In order to assist in the early diagnosis of PTLD, viral load surveillance is employed in most centers. This utility of viral load testing is discussed below and further elucidated in Chap. 6.

Tests are performed to rule out other diagnoses, as appropriate. This takes into account the likely differential diagnosis (see section "Differential Diagnoses"). Specific tests are performed to establish the histologic diagnosis of PTLD and to characterize PTLD lesions, including the presence or absence of EBV in biopsy tissue. General tests are performed to determine the presence or absence of complications of PTLD or related conditions. Depending on the nature of the tests, these are performed concurrently or sequentially.

General Tests and Non-EBV-Specific Tests

Blood Tests Initial tests include a complete blood count with white blood cell differential. In some patients with PTLD, there may be evidence of anemia which is usually normochromic, normocytic. In patients with gastrointestinal tract PTLD and occult bleeding over a prolonged period of time, there may be evidence of iron deficiency anemia with hypochromia and microcytosis. The source of bleeding can be determined by performing additional testing, namely, examination of the stools for occult blood. The blood elements may be depressed with evidence of leucopenia, atypical lymphocytosis, and thrombocytopenia. Thrombocytopenia and neutro-

penia have been shown to be associated with poorer outcomes, although the precise mechanism underlying this association is unclear [8, 9].

Depending on the location of PTLD lesions, there may be evidence of disturbance in serum electrolytes, liver, and renal function tests. Elevations in serum uric acid and lactate dehydrogenase may occur. Serum immunoglobulin levels may be elevated as part of an acute phase reaction. However, serum IgE levels have been observed to be elevated in some cases of PTLD [35]. Serum IgE levels may be elevated in the setting of a TH2 response profile which is thought be seen in patients with PTLD. The presence of elevated serum IgE may function as a proxy assay for TH2 activity. However, the relationship between PTLD and serum IgE levels has been found to be inconsistent. The presence of monoclonal or oligoclonal gammopathy has been shown to precede the detection of overt PTLD, but the specificity of this maker is poor [36].

Adjunctive tests that might predict PTLD risk or indicate the presence of PTLD have been investigated. Potential biomarkers studied include serum IL-6 [37], serum/plasma free light chains [38, 39], serum sCD30 [40], serum CXCL13 [41], and host genetic factors including HLA type [42] and polymorphisms in cytokine genes [43] but require further validation. How these markers relate to each other and to EBV viral load in predicting PTLD risk is the subject of current and future research. To date none of these markers should be definitively used for detection and follow-up.

Evaluation for the presence of cytomegalovirus is usually performed in patients with suspected PTLD. Diagnostic tests would include CMV quantitative PCR on blood as well as the examination of biopsy tissue for viral inclusions, PCR testing and immunohistochemistry for CMV. Cytomegalovirus may contribute to the net state of immunosuppression and is regarded by some experts to be a risk factor for PTLD. However, analyses of the impact of CMV disease or CMV mismatch have yielded conflicting results [44, 45]. HHV6 may also be an indirect co-factor for PTLD due to the potential for interaction with CMV [46].

Radiographic Imaging Imaging is essential in the evaluation of PTLD. Most centers employ a total body CT scan (head to pelvis) as part of the initial assessment. Beyond this, the choice of tests depends largely on the location of suspected lesions and the historical sequence of prior recent radiographic testing. Many experts recommend that a head CT or MRI be included as part of the initial workup. This is due to the fact that the presence of central nervous system lesions will influence treatment and such lesions may be solitary and may not be associated with disease in extracranial locations. CNS lesions often tend to fail therapy and are associated with high relapse rates, based on the fact that the CNS is a site that is relatively immunologically privileged.

Given the frequency of adenotonsillar involvement in PTLD, CT scanning of the neck may help to define the extent of involvement or detect subtle early changes that necessitate biopsy to rule out PTLD. Figure 8.2 shows the CT findings in a patient who was subsequently shown to have PTLD involving the adenoids. In some

patients, adenotonsillar involvement is the only site of PTLD. It is likely that at least a proportion of these asymptomatic cases with adenotonsillar involvement resolve spontaneously as immunosuppression is minimized and stabilized beyond the early months after organ transplantation.

Pulmonary lesions that are visible on chest radiographs may require high-resolution CT scanning for better delineation prior to biopsy (Fig. 8.3). Furthermore, CT of the chest may reveal mediastinal adenopathy and small pulmonary nodules that are not visible on the plain chest radiograph. Suspected intra-abdominal lesions may be evaluated with ultrasonography and CT scanning. This is in addition to other modalities of assessment, including GI endoscopy in the case of intestinal hemorrhage. Figure 8.4 shows peripancreatic and retroperitoneal node involvement in a patient with PTLD. Such findings are not specific for PTLD, and other causes of lymphadenopathy should be considered in the differential diagnosis.

PET-CT (positron emission tomography-computerized tomography) has emerged to be a useful test in the evaluation of PTLD [47, 48]. PET is a diagnostic scanning method that directly measures metabolic, physiological, and biochemical functions

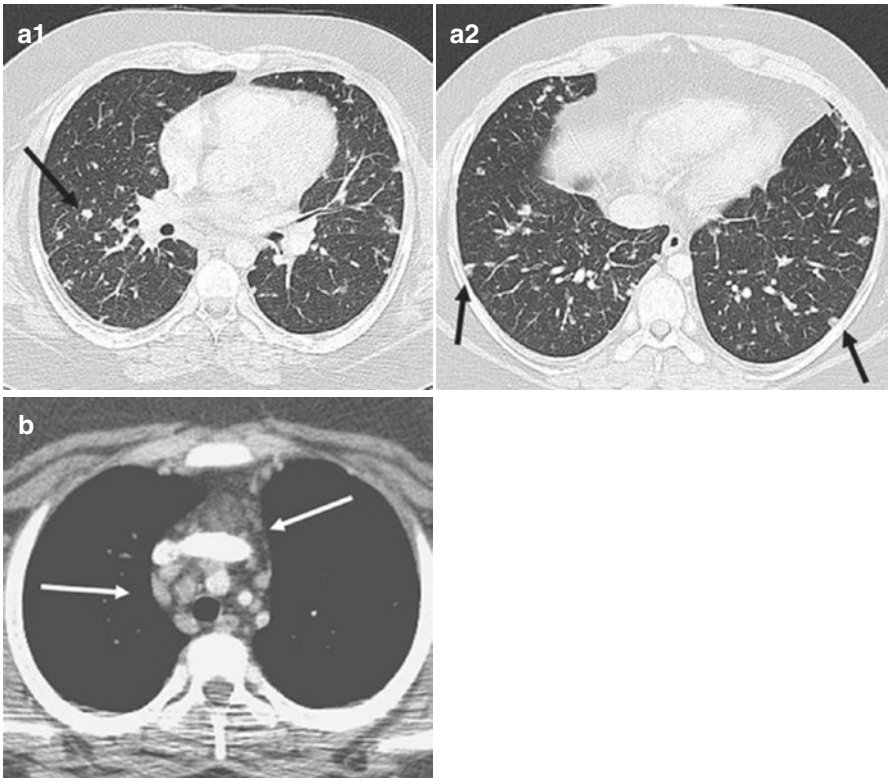

Fig. 8.3 CT (**a**) reveals multiple pulmonary parenchymal nodules and small mediastinal lymph nodes. (**b**) Biopsy of the parenchymal nodules confirmed PTLD. (Courtesy of Dr. David Manson, Hospital for Sick Children, Toronto)

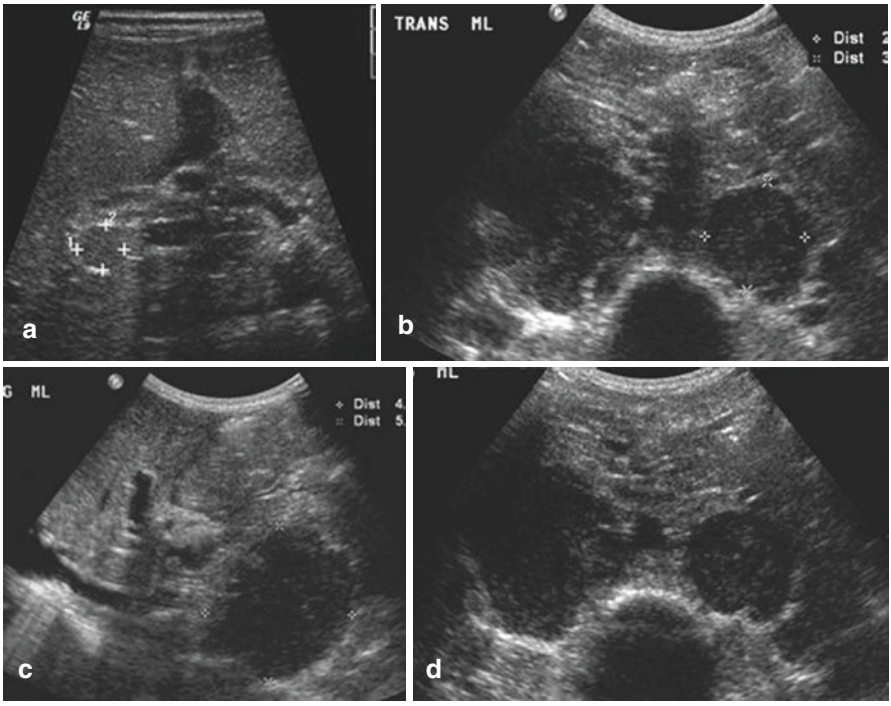

Fig. 8.4 Abdominal US of PTLD lesions: older child after HSCT and liver transplantation with elevated EBV titers. Multiple images show peri-pancreatic and retroperitoneal lymphadenopathy. (Courtesy of Dr. David Manson, Hospital for Sick Children, Toronto)

of the human body. A PET scan uses a small dose of a radionuclide combined with glucose (fluoro-2-deoxy-D-glucose, FDG) [47, 48]. The radionucleotide enables glucose metabolism to be traced, and it emits positrons, which are then detected by a scanner. Since certain tumors or lesions are known to grow at a fast rate compared to healthy tissue, the former cells will use up more of the glucose that is coupled with the radionuclide attached. The PET scan computer uses the measurements of glucose consumed to produce a color-coded picture. PET-CT utilizes a PET scanner with a computed tomography scanner in an integrated system, such that the CT provides accurate localization of lesions and the PET scan assists in interpretation of the suspected PTLD lesions. It has also proved to be of value in assessing the extent of remission after treatment (Fig. 8.5a, b). In the case of FDG-avid lymphomas, 18F-FDG-PET-CT has become the standard to assess treatment response [18, 49, 50]. Data in PTLD patients are limited and are largely confined to reports from single centers, where PET-CT has been used in diagnosis and more selectively in the follow-up of PTLD. However, a report from a registry of adult PTLD cases reported that end of treatment PET-CT had a 92% negative predictive value for disease relapse [51]. A major disadvantage is the amount of radiation delivered by PET-CT, which makes it difficult to make all-encompassing recommendations for all patients.

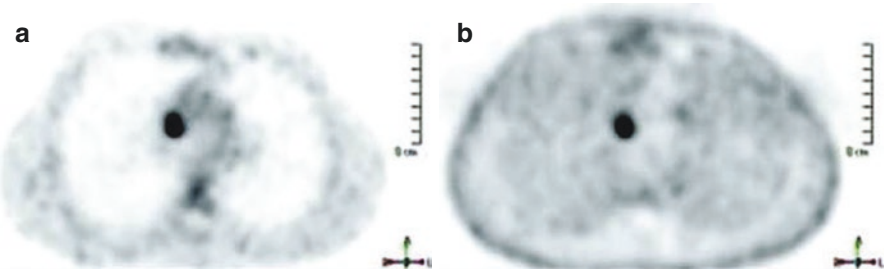

Fig. 8.5 Pretreatment PET-CT (**a**) reveals FDG-avid right paratracheal lymph node in a teenager after lung transplantation. Posttreatment study (**b**) reveals resolution of the FDG avidity and diminution of the node. (Courtesy of Dr. David Manson, Hospital for Sick Children, Toronto)

Once the diagnosis of PTLD has been determined, or is highly suspected, additional diagnostic tests may be performed to assist in defining the extent of disease. These investigations may include but are not limited to a bone scan, a bone marrow biopsy, and a lumbar puncture to assist in ruling out bone, bone marrow, and CNS disease, respectively.

EBV-Specific Tests

EBV Serology In immunocompetent patients, primary EBV infection can be determined by measuring EBV antiviral capsid antigen IgM and IgG antibodies, anti-early antigen (EA), and anti-Epstein-Barr nuclear antigen. Persistence of anti-EA antibodies has been previously shown to be more likely in PTLD patients [52], and patients who are known to be seropositive before transplantation may have falling anti-EBNA-1 titers in the setting of elevated EBV loads and the presence of PTLD [53]. However, experience has shown that serology is unreliable as a diagnostic tool for either PTLD or primary EBV infection in immunocompromised patients. These patients show a marked delay in their humoral response to EBV antigens, and many fail to develop immunoglobulin (Ig) M antibodies altogether. Another important drawback is that these patients may have received blood or blood products with the passive transfer of antibodies that render EBV IgG antibody assays difficult to interpret. In the above context, the most important role of EBV serology in the setting of transplantation is the categorization of serostatus of donors and recipients in order to determine the likely risk of PTLD.

Detection of EBV Nucleic Acids or Protein in Tissue It has been determined that 85–90% of PTLD lesions are EBV-positive. In situ analysis of biopsy specimens by polymerase chain reaction, viral antigen [54], and EBV-encoded small nuclear RNA (EBER) [54, 55] are of value in the diagnosis of EBV-associated PTLD. These modalities establish the presence or absence of EBV in the PTLD lesions. Polymerase chain reaction detection of EBV DNA in tissue is more useful in ruling out the presence of EBV in lesions than in indicating its presence as it is difficult to determine if the DNA is originating in the specific tissue as opposed to being deposited in the tissue by passenger lymphocytes. Immunohistochemistry staining may

indicate the presence of viral genes, such as LMP-1. In situ hybridization for EBER labels EBV-encoded early RNA transcripts in infected cells. This is a rapid and reliable approach that is performed in most transplant centers.

Viral Load Determination in the Peripheral Blood Technical aspects of the measurement of Epstein-Barr virus load are addressed in detail in Chap. 6. This test was first shown to be of value in the surveillance for PTLD as a result of work by Rocchi et al. [56], who indicated a relationship between PTLD and the number of EBV-infected cells in the peripheral blood. In 1994, Riddler et al. [57] and Savoie et al. [58] independently reported that an abnormally elevated EBV DNAemia correlated with PTLD development. Data from the Riddler et al. study indicated that using semi-quantitative polymerase chain reaction (PCR), patients with PTLD had a viral load greater than 5000 EBV genome copies/10^6 PBMC [57]. Other studies confirmed this relationship between viral loads and PTLD [59–64]. An association between PTLD and EBV detection in plasma has also been reported along with an increased specificity of plasma viral load in the diagnosis of EBV-positive PTLD [65]. These studies have advocated for the establishment of a threshold value for EBV DNAemia to distinguish patients at high risk for PTLD from those at low risk. The characteristics of this test as a diagnostic indicator of the presence of PTLD indicate that it is more useful in ruling out PTLD than in indicating its presence, in keeping with a low positive predictive value and a high negative predictive value.

Serial measurements of EBV load are more useful than single values. The addition of complimentary tests might increase the overall utility of viral load in the diagnostic evaluation of PTLD. In the future, these tests might include EBV-specific cytotoxic T lymphocyte measurements with or without the integration of cytokine/chemokine or viral gene expression profiling, using quantitative real-time reverse transcription-PCR and/or microarray technology.

Patients with asymptomatic sustained high loads (chronic high load carriers) require monitoring, as a proportion of these patients' clinical course evolves into PTLD. In this situation, the risk is represented by the sustained load as opposed to a specific quantifiable viral load threshold. Pediatric heart transplant recipients followed by intestinal recipients are more likely than their liver and kidney counterparts to develop PTLD in the setting of chronic high viral load carriage [66–68]. Data from prospective studies are needed to confirm these observations. In HSCT patients chronic high viral load carriage is not a frequent occurrence in the absence of chronic graft versus host disease with the resulting need for ongoing immunosuppression.

Histopathology

The pathologic examination of biopsy material is the gold standard for the diagnosis of PTLD. This is discussed in detail in Chap. 2. The presence of certain features in the lesions might assist in indicating malignant transformation and prognosis. Such criteria include monoclonality, oncogene rearrangements, and presence of specific mutations. Depending on the location of lesions, particular procedures may be

needed to obtain tissue for histopathologic examination to rule out non-PTLD diagnoses, establish the diagnosis of PTLD, and characterize PTLD lesions. These procedures may include transbronchial biopsies; surgical biopsies of internal organs, skin lesions, tissues, or lymph nodes; CT-guided needle biopsies; and endoscopic gastrointestinal biopsies, as indicated.

Clinical Staging of PTLD

No staging system currently exists for PTLD, and no single system total captures the full spectrum of what is classified as PTLD. Most centers use systems that have been developed for lymphoma staging in immunocompetent hosts, the Lugano classification system in adults [69] and the International Pediatric Non-Hodgkin Lymphoma Staging System (IPNHLSS) in children [70]. The need for routine bone marrow biopsy and lumbar puncture for staging, particularly in the absence of symptoms or signs of involvement at these sites is uncertain; routine bone marrow biopsies are not recommended in immunocompetent hosts with DLBCL if PET-CT is performed [18].

At the very minimum, staging should document the presence or absence of symptoms, the precise location of lesions, the involvement of the allograft, and the presence or absence of CNS involvement. A simple clinical categorization of lesions based on location has been proposed [14]. In EBV-positive PTLD, the virologic status should be categorized as reflected by the level of viral load. While, conceptually, an increase in load from "remission levels" after therapy may be an indicator of relapse following successful initial treatment of PTLD, this may not be consistently accurate and notably so after rituximab-based treatment [71].

Differential Diagnoses

Some conditions may mimic PTLD depending on the nature of the presenting symptoms and the location of lesions. Rejection may be confused with PTLD affecting the transplanted organs [43]. This is an important consideration, given that the former requires augmentation of immunosuppression, while reduction in immunosuppression is required in the management of PTLD.

The presence of nonspecific constitutional symptoms might suggest the presence of an infectious etiology. Critically ill patients with an acute fulminant presentation may be confused with those with sepsis. Such patients may need to be empirically treated for infections other than EBV, while the diagnosis of PTLD is being established.

Patients presenting with pulmonary nodules might have a variety of conditions that can cause these lesions, including infections due to *Mycobacteria tuberculosis*, atypical mycobacteria, *Nocardia*, *Actinomyces*, and fungal species, among other

pathogens. In lung transplant patients in particular, the differential diagnosis of pulmonary lesions includes *Aspergillus*. This deserves special mention, as in cases of pulmonary aspergillosis, careful consideration has to be given to the safety of using CT-guided needle biopsies to obtain tissue. These procedures are generally safe to do if the lesions are PTLD but may result in life-threatening pulmonary hemorrhage, if the lesions are due to *Aspergillus* [72]. In hematopoietic stem cell transplant recipients, the differential diagnosis includes graft versus host disease, particularly when the lesions are less well circumscribed with more diffuse involvement of lung parenchyma.

The differential diagnosis of lymphadenopathy includes the above entities as well as other condition causing localized or generalized lymphadenopathy. Examples include, but are not limited to, infections caused by *Bartonella* species and *Toxoplasma gondii* [73–75].

Patients with gastrointestinal symptomatology, such as diarrhea, may have a variety of other diagnoses other than PTLD. This can be particularly problematic when these symptoms occur in the setting of elevated EBV viral loads. In some patients with EBV enteritis, the boundaries of separation of this entity from PTLD can be blurred. Conditions to rule out besides PTLD or EBV disease include de novo bowel lymphomas, adenoviral disease, rejection in intestinal transplant patients, graft versus host disease in HSCT patients, cytomegalovirus disease, *Clostridium difficile* infection, intestinal mycobacterial infection and other infectious etiologies, and medication-induced diarrhea (in particular mycophenolate mofetil).

Clinicians should always be reminded that non-EBV-related malignancies may arise in the post-transplant period and enter into the differential diagnosis of PTLD [73–77]. These malignancies may be classified into three categories: preexisting recipient malignancies, de novo malignancies originating in the recipient, and donor-transmitted malignancies. These entities are generally more frequently seen in adult patients compared with children. The skin represents the most frequently documented site of involvement by these non-PTLD malignancies. A detailed discussion of these is beyond the scope of this chapter.

In disseminated PTLD, the extent of hemophagocytosis can be significant enough to create a syndrome that mimics hemophagocytic lymphohistiocytosis (HLH) [78, 79]. The latter is characterized by fever, splenomegaly, jaundice, and the pathologic finding of hemophagocytosis (phagocytosis by macrophages of erythrocytes, leukocytes, platelets, and their precursors) in the bone marrow and other tissues. Epstein-Barr virus infection is one of the etiologic agents that have been linked with HLH, even if the patient does not have PTLD. This gives rise to diagnostic confusion between PTLD with some elements of hemophagocytosis and HLH that is driven by Epstein-Barr virus in the absence of PTLD. Treatment of the latter includes, but is not limited to, chemotherapy with etoposide and dexamethasone, while the former requires reduction in immunosuppression as discussed elsewhere in this publication.

Ten Take-Home Pearls

- Early detection of PTLD is important in maximizing the chances for a successful outcome.
- Epstein-Barr virus load is more useful in ruling out PTLD than in indicating its presence.
- Epstein-Barr virus serology is unreliable as a diagnostic tool for PTLD and primary EBV infection in immunocompromised patients.
- Clinicians should have a high index of suspicion for PTLD in at-risk patients, including but not limited to those who have no pre-transplant EBV immunity.
- PTLD often affects the transplanted organ with the exception of the heart.
- Lymphoid tissues, including nodes, adenoids, and tonsils, are frequently the primary sites affected by PTLD.
- PTLD affecting the central nervous system may present as a solitary lesion.
- Knowledge of the differential diagnosis is important in preventing missed diagnoses of non-PTLD diseases.
- *Positron emission tomography-computerized tomography* has emerged to be a useful test in the evaluation of PTLD.
- Histopathologic examination is the gold standard for the diagnosis of PTLD.

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