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Epstein-Barr Virus Infection and Posttransplant Lymphoproliferative Disorder

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This document discusses the epidemiology, clinical manifestations, diagnosis, and management of Epstein-Barr virus (EBV) infection and posttransplant lymphoproliferative disorder (PTLD). It highlights the importance of EBV in solid organ transplantation (SOT) recipients, emphasizing the need for preemptive therapy and the role of EBV viral load monitoring in diagnosis and management. The development of standards for management based on EBV viral load and routine monitoring of EBV-specific CTL responses is also discussed.

Key words: Epstein–Barr virus, malignancy, organ transplantation, posttransplant lymphoproliferative disorder

Abbreviations: CNS, central nervous system; CTL, cytotoxic T lymphocytes; EBV, Epstein–Barr virus; IVIG, intravenous immune globulin; MRI, magnetic resonance imaging; NAT, nucleotide amplification test; PBL, peripheral blood leukocyte; PBMC, peripheral blood mononuclear cell; PCR, polymerase chain reaction; PTLD, posttransplant lymphoproliferative disorder; SOT, solid organ transplantation; VL, viral load.

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Introduction

Solid organ transplantation (SOT) is accepted therapy for a wide range of conditions associated with end-stage diseases of the heart, kidney, lung, liver, as well as intestine and pancreas. The success of organ transplantation is dependent upon the use of immunosuppressive agents to prevent or treat rejection of the allograft. Over time, the increasing number of therapeutic options and growing understanding of how to use them to achieve adequate control of the immune response to the allograft has led to improved patient and graft survival related to rejection. However, successful control of rejection has come at the cost of increased susceptibility to infections in the posttransplant period. This is particularly true of herpesviruses in general and Epstein–Barr virus (EBV) in particular. Because EBV is exceptionally prevalent in the general population, the possibility of developing infection and disease due to this virus is a frequent concern for recipients of organ transplantation. While EBV is associated with a wide range of clinical manifestations in SOT recipients, most attention has focused on its most serious manifestation, posttransplant lymphoproliferative disorder (PTLD). Although this complication occurs more frequently in pediatric patients experiencing primary infection (1), PTLD can arise in both adults and children undergoing SOT. While some patients may develop EBV-negative PTLD, this is not the focus of this article whose primary goal is to provide an overview of the clinical manifestations, diagnosis and management of EBV infection in SOT recipients, highlighting accepted therapies as well as future directions for treatment and prevention.

Epidemiology of EBV and EBV-associated PTLD

Epstein–Barr virus is a gamma herpesvirus which is a ubiquitous cause of infection in humans with a seroprevalence of over 90–95% of adults worldwide (2). Exposure to EBV begins early in life with approximately 50% of children in developed countries becoming seropositive by 5 years of age (2). The timing of EBV infection varies with socioeconomic status; earlier acquisition occurs in developing countries as well as in individuals coming from lower socioeconomic conditions. Most infections occurring in young immunocompetent children are subclinical or present as an unremarkable febrile upper respiratory tract infection. A second wave of infection occurs in the 15- to 24-year-old age group (2). Infection occurring in this older cohort frequently presents as the classic syndrome of infectious mononucleosis which is characterized by fever,
lymphadenopathy and hepatosplenomegaly, often with a concurrent hepatitis. The relationship of age to seroprevalence results in pediatric organ transplant recipients being at greater risk of acquiring primary EBV infection compared to adult recipients at the time of transplant. Exposure to EBV after transplant may occur by way of passenger leukocytes from an EBV seropositive organ donor, through blood products or via typical exposures occurring also in immune competent individuals in the community. As primary EBV infection is a major risk factor for the development of symptomatic EBV disease including PTLD, pediatric organ recipients are generally at greater risk of developing these complications than adults undergoing organ transplantation (3).

The overall prevalence of EBV-associated PTLD following SOT ranges from 1% to 20%, with rates varying according to the type of organ transplanted, pretransplant EBV serostatus and the age of the recipient (1,4,5). EBV-positive PTLD typically present relatively early after transplant with the highest incidence occurring in the first year after transplant although later cases do occur. The reported rates of EBV-associated PTLD underestimate the total burden of disease attributable to EBV as data defining rates of symptomatic EBV disease not meeting the diagnostic criteria for PTLD are infrequently reported. The variation in rates of PTLD observed in recipients of differing allografts likely reflects the different levels of immunosuppression required to maintain these organs, though other factors such as the amount of lymphatic tissue within the allograft may also contribute to varying risk.

A growing number of cases of EBV-negative PTLD have been reported. In general, EBV-negative PTLD are seen more frequently in adult compared to pediatric SOT recipients. These cases tend to present later (> 5 years after transplant) and to a large extent account for the observed bimodal pattern of timing of presentation of PTLD, with early cases being predominantly EBV-positive and late cases being increasingly EBV-negative (6,7). Recent data from a French Kidney Transplant Registry suggest that an increasing number of cases of EBV-negative PTLD present between 7 and 10 years after transplant (6). Data from the pediatric heart transplant registry likewise suggest that EBV-negative PTLD is increasingly being diagnosed in children presenting late after transplant. A broader discussion of EBV-negative PTLD is beyond the scope of this review.

Organ specific 1- and 5-year cumulative incidence rates of PTLD (combined data from both EBV positive and EBV negative lesions) as reported to the Organ Procurement Transplant Network for adult and pediatric recipients are shown in Table 1. In general, for a given organ type, rates in pediatric recipients exceed those in adults due to the increased incidence of pediatric recipients without prior EBV infection at the time of transplant (8,9). The highest rates of PTLD have been reported in recipients of intestines and lungs with lower rates for recipients of livers, hearts and kidneys (10,11). Regardless of graft type, improved detection and management of EBV infection in SOT recipients has led to a reduction in PTLD rates over time. This is at least in part due to earlier recognition of EBV infection at a point where it is either subclinical or where disease associated with this pathogen does not meet diagnostic criteria for PTLD. Despite these improvements, the risk of developing PTLD in seronegative patients experiencing primary EBV infection early after transplant remains significant.

While reported mortality rates associated with EBV/PTLD are quite variable, two trends are notable: mortality is improving across allograft types after PTLD, and children do far better than adult organ recipients. Estimates of 1-year survival after PTLD diagnosis run from 56% to 73%, with 5-year estimates falling between 40 and 61% (7,12–14). Further, outcome of EBV-positive PTLD appears to be superior to EBV-negative disease as is survival for patients presenting with PTLD early (<2 years) compared to late (> 5 years) after transplant (7). The presence of central nervous system (CNS) involvement with PTLD has also been shown to be associated with worse outcome. Of note, the use of rituximab-based therapy in conjunction with reduced immunosuppression has been shown to improve survival in at least one series of PTLD in adult SOT recipients (15), though conclusive data confirming this observation from large randomized trials are not available.

Pathogenesis of EBV Infection

Biology of EBV infection in the immunocompetent host

Epstein–Barr virus was first identified in the tissue of a patient with Burkitt’s lymphoma, and its association with transformed and proliferating lymphocytes is well recognized (16). Similar to other herpesviruses, its life cycle is characterized by a lytic phase where it can infect other cells or spread infection to naive individuals and the more quiescent latent phase where it persists lifelong in memory B cells. Under normal circumstances the virus is transmitted from one person to another through saliva containing infectious virions and enters via the oral pharynx. Whether EBV enters the host through pharyngeal epithelium or naive B cells in the submucosal lymphoid

Table 1: Cumulative 1- and 5-year incidence of PTLD in pediatric and adult SOT recipients by transplanted organ as reported in the 2010 OPTN/SRTR Annual Report (97)*

<table>
<thead>
<tr>
<th>Organ</th>
<th>Pediatric 1 year</th>
<th>Pediatric 5 years</th>
<th>Adult 1 year</th>
<th>Adult 5 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung/heart-lung</td>
<td>4.0%</td>
<td>16%</td>
<td>1.0%</td>
<td>1.5%</td>
</tr>
<tr>
<td>Liver</td>
<td>2.1%</td>
<td>4.7%</td>
<td>0.25%</td>
<td>1.1%</td>
</tr>
<tr>
<td>Pancreas (isolated)</td>
<td>N/A</td>
<td>N/A</td>
<td>2.3%</td>
<td>2.3%</td>
</tr>
<tr>
<td>Heart</td>
<td>1.6%</td>
<td>5.7%</td>
<td>0.3%</td>
<td>0.7%</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.3%</td>
<td>2.4%</td>
<td>&lt;0.2%</td>
<td>0.6%</td>
</tr>
</tbody>
</table>

*Data for intestinal transplant recipients not broken down by pediatric versus adult and therefore not included.

Green and Michaels

EBV and PTLD

layer has been an area of ongoing debate but eventually the virus infects the host’s B lymphocytes establishing a reservoir of latent virus (2).

Viral gene expression varies according to the phase of EBV viral infection. The lytic phase predominates during early infection leading to destruction of host cells and release of progeny virions. A wide variety of viral proteins are produced and expressed in this phase of infection with BZLF1 and BRFL1 being key to activating lytic replication (2). The latent phase is characterized by expression of less antigens and its ability to immortalize infected B cells. A variety of interactions occur during latency between viral miRNA and proteins with the infected cells resulting in modulation of apoptosis and other B cell functions which promote the maintenance of the infected latent state (17).

In the immunocompetent host, early infection generates a brisk immune response from cytotoxic T lymphocytes (CTL) with as many as 40% of all circulating T lymphocytes at this point directed against EBV (18). As the host CTLs establish control, the lytic phase of infection recedes and latent infection of the memory B lymphocytes predominates. EBV infection persists for life and maintains a state of constant tension between the host immune response and latent virus (1 in every 10^6 B cells in most adults) punctuated by some cells being permissive for lytic viral shedding (19).

**EBV proliferation under immune suppression**

For organ recipients, the virus can cause infection via latently infected donor B cells traveling within the graft. The increased risk of PTLD in recipients of lymphoid-rich organs such as intestine and lung may in part be due to the amount of infected donor B cells coming over with the graft. The host’s ability to control EBV after primary infection is reliant on a functional T cell repertoire. Accordingly, primary infection after SOT can be hampered by the immunosuppressive medications that specifically target T cell function. Despite this, transplant recipients are generally able to mount some level of specific immunity to EBV. However, compared to the normal host the development of antiviral immune response is decreased or incomplete and the reservoir of latently infected B cells is larger (20). In addition, they may not be able to develop an adequate population of EBV-specific cytotoxic CD8+ T lymphocytes (EBV-CTL). One of the best predictors of the ability to control EBV infection is the presence of an EBV-CTL with studies in both recipients of SOT and hematopoietic stem cells demonstrating the level of EBV-CTL activity correlating well with both the magnitude of circulating EBV, and the likelihood of developing PTLD (21,22). In general, the less immunosuppressed the patient, the better the CTL response and the less likely the recipient is to develop PTLD (23,24). Patients undergoing lung or intestinal transplants often require more intensive immunosuppression decreasing their ability to have an effective EBV-CTL response and explain-

ing in part their increased risk for EBV disease and PTLD. Similarly, the use of induction treatment or treatment of rejection with T cell-specific immunosuppressive agents negatively influences the development of EBV-CTLs after primary infection as well as to protect against EBV in a recipient who is previously immune. Among induction agents, the use of anti-T lymphocyte antibody preparations such as OKT3 and thymoglobulin is associated with increased risk for the development of PTLD (25–27).

While some clinicians have expressed a concern that the use of tacrolimus-based immune suppression was associated with an increased risk of PTLD compared to cyclosporine base regimens, an evaluation of OPTN data for adult kidney transplant recipients did not show this to be the case (27). However, the use of m-TOR inhibitors was associated with an increased rate of PTLD in this analysis. More recently, the use of belatacept as maintenance therapy as an alternative to chronic calcineurin inhibitor use for adult kidney transplant recipients was found to have unexpectedly high rate of EBV-associated PTLD (especially involving the CNS) in EBV seronegative recipients leading to a specific contraindication its use in this population (28–30). This clearly emphasizes the need to continue to assess the impact of newer immunosuppressive agents and regimens on EBV disease as part of the formal evaluation of new agents and combinations.

**Spectrum of EBV Disease**

Clinical manifestations of EBV infection range from asymptomatic infection to clinically significant and potentially life threatening disease in SOT recipients. EBV infection can be either primary (new infection occurring in an immunologically naive patient) or secondary due to either reactivation of latent EBV in the transplant recipient under the pressure of immune suppression or reinfection with a new EBV strain. In general, primary infection is associated with more clinically significant disease while secondary infection tends to be mild or even asymptomatic (3). The spectrum of clinical disease includes a nonspecific viral syndrome, mononucleosis and PTLD including EBV-associated malignant lymphoma (e.g. Burkitt’s lymphoma). Rarely, EBV has been associated with posttransplant smooth muscle tumors as well (31). Of interest, these frequently occur in patients with a prior history of PTLD. EBV positive T cell PTLDS are also rare occurrences and are associated with a very poor prognosis.

Histologic evaluation is important in defining disease status of a patient with suspected PTLD; manifestations can evolve in individual patients (1). The World Health Organization has provided standardized criteria for the pathologic evaluation of lesions associated with EBV in SOT recipients (32). However, histologic grade of multiple lesions obtained simultaneously from the same patient can vary potentially limiting the accuracy of pathologic assessment.
(33). Clinical criteria defining the spectrum of EBV disease not meeting histologic definition of PTLD have also been proposed (4). While the application of a classification scheme for EBV-related disease is useful, it is important to note that EBV presents as a continuous spectrum of illness, and benign manifestations can evolve to more serious syndromes within individual patients. Furthermore non-PTLD viral syndromes are not always benign, and fatal viral sepsis may occur in the absence of mass lesions.

Variation in severity and extent of disease is felt to be related to the degree of immunosuppression and adequacy of the host immune response. Symptomatic EBV infection and PTLD in particular are more common after primary EBV infection, thus affecting children disproportionately. In one study, 4% of children undergoing (SOT) and 10% of children with primary EBV infection developed PTLD between 1 month and 5 years after transplant; 75% of cases occurred during the first postoperative year in patients receiving cyclosporine-based immunosuppression (8). Cumulative occurrence can reach as high as 12–20% by 7–12 years after liver transplantation (34,35). Onset of viral syndrome, mononucleosis and polymorphic PTLD occur primarily within the first year, whereas monomorphic PTLD and lymphoma tend to occur later.

**Diagnosis of EBV Infection and PTLD After Transplantation**

As noted above, EBV infection can be asymptomatic, present with signs and symptoms of typical infectious mononucleosis, or with clinical signs and symptoms related to the specific organ system involved. The availability of nucleic acid amplification tests (NAT) such as PCR of the peripheral blood looking quantitatively at viral load has revolutionized the ability to monitor and help diagnose EBV infection and PTLD. Strategies of EBV viral load (VL) monitoring have become a routinely used powerful tool for detection of EBV and estimation of the risk for development of PTLD. Although specific controlled trial data are lacking for many of the uses of EBV VL testing, published guidelines support the routine use of the viral load to guide therapeutic choices for EBV infection, immunosuppression and management of PTLD (1).

Interestingly, the initial experience in EBV viral monitoring in SOT recipients was not with peripheral blood but rather with oropharyngeal shedding, noting increased shedding of EBV associated with clinically significant EBV-driven disease in SOT recipients (36). Ultimately this was found to be a less reliable way to predict PTLD risk compared to assessing quantitative viral loads in peripheral blood using NAT; this coupled with its labor intensive nature led to it losing favor as a diagnostic tool (37). Since that time, refinement of the measurement of EBV VL has occurred both with advances in quantitative PCR technology, as well as collection of large serial data sets to determine relative values of EBV VL which might predict the development of PTLD (38,39). While monitoring is routine for the high-risk groups such as seronegative recipients, especially those receiving organs from seropositive donors (EBV mismatched), several areas of controversy deserve discussion. First it should be noted that the optimal component of the peripheral blood to test is not fully defined with conflicting results for assays using peripheral blood lymphocytes, whole blood or plasma (1,38,40–42). Peripheral blood lymphocytes and mononuclear cells (PBL/PBMC) contain EBV within infected B cells, either typical memory B reservoir cells, or aberrant Ig-null phenotype cells which harbor much higher genome copies (43). Conversely, serum and plasma sampling measure the presence of viral DNA, either contained in mature virions or as fragments, which are more common in acute infection or EBV-driven malignancies (38). Whole blood sampling has been examined to minimize sample preparation, and EBV VL measurements from whole blood correlate well with PBL/PBMC levels but not with plasma/serum loads (42). While the specific compartment to assay and the precise cutoff to use for detection remain contentious, there is general agreement that EBV VL is higher in seronegative patients that develop EBV/PTLD than in those with asymptomatic EBV conversion (40). Another major limitation has been the fact that EBV VL monitoring has not been standardized between laboratories. This was highlighted by Preiksaitis et al. in a study in which known samples were sent to a large number of blinded laboratories who routinely performed EBV VL monitoring; while individual centers demonstrated a high level of internal reproducibility, substantial variability was found between laboratories (44). This poor interlaboratory reproducibility contributes to a lack of consensus on threshold EBV VL which should trigger diagnostic and therapeutic interventions. Accordingly, for individual patients, assays should be performed at one specific site even when they leave the transplant center. In addition, research studies using EBV VL as an end point should have a reference laboratory for appropriate interpretation (45). It is hoped that the recently released first WHO International Standard for Epstein–Barr Virus for Nucleic Acid Amplification Techniques will allow for enhanced standardization which may overcome some of the current concerns noted above (46).

While viral load testing has led to improved monitoring for EBV infection, it alone cannot be used to diagnose PTLD as the test can lack both sensitivity and specificity. At times the viral load will remain low if the site of PTLD is protected such as early in the graft itself or in some gastrointestinal lesions while patients with elevated EBV VL do not always have or develop EBV/PTLD. For these reasons an aggressive approach to the evaluation of PTLD should be used when this diagnosis is suspected. Consideration for PTLD should be high in the presence of any unexplained febrile illnesses in a SOT recipient, particularly those in the first year after transplantation or who have had augmented immune suppression for treatment of rejection. PTLD should also be considered in any patient with an elevated EBV
Table 2: Signs and symptoms of posttransplant lymphoproliferative disorder

<table>
<thead>
<tr>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Constitutional and systemic symptoms:</strong></td>
</tr>
<tr>
<td>Unexplained fever or night sweats</td>
</tr>
<tr>
<td>Malaise</td>
</tr>
<tr>
<td>Weight loss and/or anorexia</td>
</tr>
<tr>
<td>Sore throat</td>
</tr>
<tr>
<td>Swollen glands</td>
</tr>
<tr>
<td>Headache or focal neurologic symptoms</td>
</tr>
<tr>
<td><strong>Allograft-specific symptoms</strong></td>
</tr>
<tr>
<td>Liver: jaundice, abdominal pain</td>
</tr>
<tr>
<td>Intestine: abdominal pain, gastrointestinal bleeding, nausea and vomiting</td>
</tr>
<tr>
<td>Heart/lung: shortness of breath, cough, decreased lung function (lung alone)</td>
</tr>
<tr>
<td>Renal: kidney dysfunction</td>
</tr>
<tr>
<td><strong>Mass lesions found on imaging obtained for other reasons</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pallor</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
</tr>
<tr>
<td>Subcutaneous nodules</td>
</tr>
<tr>
<td>Tonsillar enlargement or inflammation</td>
</tr>
<tr>
<td>Hepatosplenomegaly</td>
</tr>
<tr>
<td>Focal neurologic signs</td>
</tr>
</tbody>
</table>

VL and focal findings on examination or in a patient with primary EBV infection and increasing viral loads. Many signs and symptoms of PTLD are shared with more typical EBV infection including pharyngitis, tonsillar enlargement, lymphadenopathy, or hepatosplenomegaly (see Table 2). Lymphoma may present with focal lesions in any organ, although the allograft itself is a common site for the development of PTLD (25). The gastrointestinal tract is frequently a site of involvement regardless of the organ transplanted because of its rich amount of lymphoid tissue and can manifest as diarrhea with blood and abdominal pain. Perforation of the intestine with a ‘volcanic’ appearing eruption is not infrequent. Central nervous system involvement can be found in 4–15% (47) and less frequent manifestations such as dermatologic changes have also been reported.

Radiographic evaluation using CT of neck, chest and abdomen may identify lesions not apparent from symptoms or examination (1,3) and should be performed when PTLD is suspected or found to allow for staging. Magnetic resonance imaging (MRI) of the brain is paramount if there are any central nervous system symptoms such as headache, focal neurologic findings or visual changes. Some experts advocate routine MRI or CT of the head in all patients at the time of initial imaging particularly in children to identify asymptomatic lesions (1). Biopsy of lesions or sites of disease is needed to definitively diagnose PTLD and rule out other opportunistic infections that might require alternate therapy or be present concurrently. Because the bowel can frequently be involved in PTLD, early endoscopy and colonoscopy should be performed in patients with unexplained abdominal pain and diarrhea. In addition, recipients of intestinal grafts may manifest similar symptoms with rejection or infection with other pathogens.

Biopsy specimens should be evaluated by a pathologist familiar with PTLD and specific assays should be performed to characterize the involved cell with emphasis on measurement of EBV-infected cells (48). A suggested list of studies to be obtained in the workup of PTLD is noted in Table 3.

As previously noted, measurement of the EBV VL of the blood alone has limitations not only in making the initial diagnosis but also when used for follow-up and prediction of recurrent disease. Accordingly interest has been directed to adjunctive testing assays which might enhance the performance of the EBV VL. Since the development of PTLD represents an imbalance between the host’s immune response and viral-driven proliferation of immortalized B cells, attention has focused on measurement of EBV CTL response. A provocative study in pediatric liver transplant recipients looked concurrently at EBV loads and EBV CTL activity using ELISPOT; the investigators found a 100% positive predictive value for the development of PTLD in recipients who experienced primary EBV infection without developing a significant EBV CTL response (21,49).

Table 3: Diagnostic workup for suspected PTLD

<table>
<thead>
<tr>
<th>Routine</th>
<th>Selected patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBC, differential, platelets,</td>
<td>Gastrointestinal endoscopy</td>
</tr>
<tr>
<td>Serum electrolytes, calcium, BUN and</td>
<td>Bone scan</td>
</tr>
<tr>
<td>creatinine</td>
<td></td>
</tr>
<tr>
<td>Liver function tests</td>
<td>Bone marrow biopsy</td>
</tr>
<tr>
<td>Uric acid</td>
<td>Brain CT/MRI</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>Lumbar puncture</td>
</tr>
<tr>
<td>Quantitative immunoglobulins</td>
<td></td>
</tr>
<tr>
<td>EBV serologies (anti-EBNA, VCA and EA)</td>
<td></td>
</tr>
<tr>
<td>EBV viral load from peripheral blood</td>
<td></td>
</tr>
<tr>
<td>Stools for occult bleeding</td>
<td></td>
</tr>
<tr>
<td>Chest radiograph (anteroposterior and lateral)</td>
<td></td>
</tr>
<tr>
<td>CT scan of neck/chest/abdomen/pelvis</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Diagnostic workup for suspected PTLD

1 Some centers are now using PET scans though the full utility of these for diagnosis and staging of PTLD remains to be determined.

2 This is not routinely performed at all centers.

Abbreviations: EBNA = Epstein–Barr nuclear antigens; EA = early antigen; VCA = viral capsid antigen.

EBV and PTLD


45
or high EBV VL and found that those with a persistently high EBV VL had a low CTL response on the basis of an ‘immune exhaustion’ phenotype which they felt predisposed these patients to PTLD (49). While measurement of EBV-specific CTL appears promising as a clinically helpful adjucnt marker, current technology does not appear to allow for routine clinical use of these assays.

Attention has been drawn to a number of additional candidate markers as potential adjunct assays to the EBV load. While previous candidate markers (e.g. mRNA for LMP2a) have not been successfully validated, newer options including free light chains, sCD30, IL-6, CXCL13 and NK cells are of current interest. Future data will hopefully clarify which if any of these candidates markers might rise to the level of being of clinical value.

Management of EBV and PTLD

Starzl and colleagues first reported on the therapeutic role of reduction or cessation of immunosuppression in the management of PTLD in 1984 (51). While nearly 30 years of experience have confirmed the general efficacy of this approach, this strategy appears to fail in ∼35–40% of pediatric patients with PTLD either due to tumor unresponsiveness or significant rejection (1,52) and is less efficacious in adult recipients with PTLD (53). This is particularly true for PTLD lesions that are no longer under the control of EBV and behave more like malignancies. Accordingly, other modalities are needed. While a number of other therapeutic strategies have been proposed, published experience to date with these has been limited to case reports and small series without randomization. Accordingly, the therapeutic efficacy of and role that most of these options should play in the management of EBV/PTLD remain unclear. In addition, because PTLD represents a heterogeneous spectrum of disease it is likely that some treatment modalities (or combination of treatments) will be most effective for specific stages of PTLD but not others. In recent years attention has focused on several newer strategies, including the use of the anti-B cell antibody rituximab, low-dose chemotherapy and adoptive immunotherapy (which has been used successfully in recipients of hematopoietic stem cell transplant recipients). A review of the main strategies employed in the management of PTLD follows.

Reduction of immune suppression

Reduction or cessation of immune suppression has been used for several decades as a first line approach to manage EBV/PTLD (1,51,52). This strategy is based on the hypothesis that recovery of the host’s immune system will allow for the development of CTL against EBV with subsequent control of EBV-driven B cell proliferation. Using this approach alone or in combination with other strategies, successful regression of both polyclonal and monoclonal EBV-associated PTLD lesions has been reported to occur in 23–86% of patients (52). The wide variation in reported response may be explained by differences in the definitions and heterogeneous nature of PTLD as well as the amount and duration of reduction of immune suppression employed. The majority of patients in whom this strategy will succeed demonstrate some evidence of clinical response within 2–4 weeks of reduction of immune suppression, though a belated response has been observed. Reduction of immune suppression is unlikely to be effective against PTLD lesions that are no longer under the control of EBV and behave more like true malignancies. At the Children’s Hospital of Pittsburgh of University of Pittsburgh Medical Center reduced immune suppression is attempted first unless there is concurrent rejection or histological evidence of true malignancy.

Antiviral therapy

Hanto and colleagues first reported on the benefit of acyclovir chemotherapy for treatment of EBV/PTLD in a case report of a patient whose EBV-associated PTLD lesion came and went in association with starting and stopping this antiviral agent (54). Acyclovir and ganciclovir inhibit lytic EBV DNA replication in vitro; ganciclovir has approximately eightfold greater potency in vitro and has the additional advantage of inhibiting CMV, a potential copathogen. However, neither agent has in vitro activity against EBV latently infected B cells nor have they been effective in treating healthy individuals with acute EBV infection (55). The majority of EBV infected cells within PTLD lesions are transformed B cells that are not undergoing lytic infection (43). Furthermore, EBV viral loads in the peripheral blood can climb to very high levels and PTLD may develop while patients are receiving intravenous acyclovir or ganciclovir (56). Despite this, most transplant centers use acyclovir, ganciclovir or their respective bioavailable oral formulations as routine adjunctive therapy based on the early reports, as well as a general comfort with the use of these agents and their theoretical benefit against lytic EBV populations in vivo. The efficacy of these agents however has not been established in prospective, comparative clinical trials and their role in the treatment of EBV/PTLD has been questioned.

Two additional potential agents warrant mentioning. Arginine butyrate has been proposed as a potential antiviral agent to be used in combination with ganciclovir or acyclovir. Arginine butyrate induces the switch from latency to lytic phase in EBV-infected lymphocytes, thereby making infected cells susceptible to the effects of these agents (57). A recently completed phase 1/2 trial evaluated the safety and tolerability of arginine butyrate in combination with ganciclovir in EBV-associated lymphoid malignancies and lymphoproliferative diseases in 15 patients who had failed prior chemotherapy or radiotherapy including some with early-onset PTLD. Although preliminary in nature and limited in duration of treatment, 4 and 6 of the 15 patients demonstrated complete response and partial response, respectively. Unfortunately, this agent is no longer available.
The second (though less well developed) novel strategy involves the potential use of bortezomib, a proteasome inhibitor approved for the treatment of myeloma and mantle cell lymphoma (58). Though results from different laboratories have been variable, in vitro data appear to demonstrate that bortezomib also induces EBV lytic activation making it a potential alternative candidate to be used in combination with ganciclovir as treatment for EBV-associated PTLD. To date, there are no published studies or case reports in support of this approach.

**Interferon**

In the 1980s and 1990s various forms of interferon were employed for individuals with recalcitrant PTLD based on their proinflammatory role as well as the absence of other treatment options. Anecdotal reports showed some success (59,60). While theoretical support for this strategy exists, concern for rejection and the availability of other treatment options led to a decreased enthusiasm for these agents in treating PTLD.

**Intravenous immune globulin**

Intravenous immune globulin (IVIG) has been considered as a potential adjunctive treatment for EBV/PTLD based on several findings. First an association between loss or absence of antibody against Epstein–Barr nuclear antigens (EBNA) and development of PTLD in infected organ recipients has been noted (61). Second early reports found a drop in EBV VL to correlate with increasing levels of antibody against EBNA even when the antibody was passively transmitted (62). In addition, case reports noted successful treatment of PTLD when using IVIG in combination with interferon-alpha (59). While no prospective systematic trials are available, these reports provided a basis for considering adding IVIG to the treatment of EBV/PTLD which has been adopted by some centers.

**Anti-B cell antibodies**

As previously noted, the majority of PTLD lesions are of B cell origin, accordingly enthusiasm for monoclonal antibodies directed against B cells has been high. Initial studies using monoclonal anti-CD21 and anti-CD24 antigens showed promise for some forms of PTLD after organ and bone marrow transplantation but are no longer available (63). In more recent years the humanized, chimeric anti-CD20 antibody, Rituximab has been available in many countries and is increasingly used in the treatment of EBV/PTLD. Single center reports and a small, multicenter study demonstrated rituximab effectiveness to be variable with a range of 28–59% when used either alone or in combination with chemotherapy for adults with PTLD that did not respond to reduced immunosuppression (64–66). Longer term follow-up in a multicenter trial of 60 patients however showed that 26% of patients who initially responded had evidence of disease progression within the first year after therapy (67). Another multicenter report appeared to demonstrate a survival advantage when rituximab was used in conjunction with reduced immune suppression (15). Studies in children appear to show better outcomes but are limited by small numbers. A small clinical trial showed an 80% initial response but a 25% relapse rate (68). A larger retrospective analysis of a French registry looked at the use of rituximab in 32 patients (27 of whom also had reduced immunosuppression) and found that 65% of treated patients who had organ transplantation went into remission. While most had sustained response, 20% had PTLD relapse a median of 7 months after treatment and follow-up was short for many (69). Despite the variability most centers remain enthusiastic about its potential value for treating PTLD particularly in those cases where reduced immunosuppression has failed or where it cannot be used due to concomitant rejection or fulminant presentation. Some centers have moved to using rituximab as first line therapy but the relapse rate noted in reports and the lack of knowledge regarding long-term toxicity associated with this treatment should lead to caution and a call for further studies.

**Chemotherapy**

The Children’s Oncology Group (COG) found a 2-year sustained response rate of 67% using a regimen consisting of six cycles of low-dose cyclophosphamide and prednisone for patients who failed reduced immunosuppression found (70). However, children who presented with fulminant PTLD did poorly with this regimen (all four died with only one partial response). COG recently completed a second study evaluating the addition of rituximab to the above strategy showing the 2-year sustained response to be similar to the prior study but noted substantial benefit for all four children with fulminant disease. In this study 55 children with CD 20+ PTLD after organ transplantation were enrolled if they failed at least 1 week of reduced immuno-suppression (33). The majority (73%) of patients in this study had monomorphic PTLD; 37 (69%) had complete remission although three relapsed. While a 1-week trial of reduced immunosuppression may not have been sufficient to determine true failure of this strategy alone, a higher than anticipated number of patients had monomorphic disease which tends to be less responsive to withdrawal of immunosuppression. Although the investigators note that this study could not determine whether the use of rituximab added benefit to the chemotherapy alone, they noted that it was generally well tolerated and the safety data were comparable to historical controls receiving chemotherapy alone.

The need for traditional chemotherapy treatment of PTLD appears to be more frequent in adults compared to pediatric organ transplant recipients. Many regimens have been used but the common strategies employ a standard dose cyclophosphamide and prednisone regimen based on treatment of adult B cell lymphomas, and achieves success in approximately two-thirds of treated patients (71). More encouraging is a recent report of the sequential
use of rituximab followed by CHOP demonstrated a complete or partial response rate of 90% (72). Future work is needed to determine the optimal chemotherapeutic regimen, confirm the potential role of combined versus sequential therapy with rituximab and the timing of instituting chemotherapy for various histological stages of PTLD.

**Radiation and surgery**
The use of surgical resection or radiation for treatment of PTLD has usually been limited to cases of isolated lesions or for debulking when making a diagnosis. In addition, surgical intervention may be required in fulminant cases when airway compromise is present due to mass effect or if gastrointestinal perforation has occurred. Since PTLD is most often a systemic disease, surgery alone is not likely to be successful long term. A recent case series of 34 patients with PTLD of the central nervous system (CNS) found a 5-year survival of approximately 50% with a reasonably high response rate to radiotherapy with or without concomitant treatments (73). Though response to chemotherapy was less successful than radiotherapy, the authors still recommended that systemic chemotherapy be considered for patients with CNS PTLD in the absence of systemic contraindications (73).

**Cellular therapy**
The final treatment strategy to review is that of cellular therapy for EBV-driven PTLD. Since the normal host uses EBV-directed CTL to control EBV infection it is logical to anticipate that EBV-specific cellular therapy would be of benefit. This strategy has been successful in PTLD after bone marrow transplantation where the tumor is generally donor derived and the donor remains available to provide T cells (74). This strategy however has not translated easily to the SOT arena, where most PTLD are of host origin requiring the presence of host EBV-specific CTLs to control the EBV-driven proliferation. Unfortunately, strategies using recipient cells have been tried but the highest risk recipients are EBV naive prior to transplant and have dysfunctional T cells after transplantation due to iatrogenic immunosuppression. Despite these limitation, attempts have been made by Savoldo et al. to “immunize” or stimulate recipient T cells against EBV ex vivo and then reinfuse them (75). The investigators reported that all 12 of their patients who received infusions of their own EBV-specific CTLs had a decline but not loss of EBV viral load. The ability to generate EBV-specific CTLs ex vivo, overcame one of the major hurdles for immunotherapy (76,77). To overcome the need to have readily available CTLs for organ transplant recipients with PTLD, Haque and colleagues in the United Kingdom used healthy blood donors to generate a bank of 100 EBV-specific CTL which would cover most of the common HLA types in the United Kingdom (78). They conducted a phase II multicenter trial using ‘best fit’ HLA match from their bank to treat PTLD after organ transplantation when other treatment modalities had failed. The EBV-specific CTL infusions were well tolerated and they achieved an overall response rate of 52% at 6 months (14/33 with complete response and three with partial response) (78). Response rate was associated with better HLA matching. Longer term follow-up was reported in 2010 showing substantial survival benefit in those who had been complete responders with 12 of 14 being alive 4–9 years later without recurrences compared to only six of 19 nonresponders who survived the initial treatment (79). Although these results are very encouraging, few centers have the technical facilities and experience to implement adoptive immunotherapy against EBV for organ transplantation at this time.

**Prevention of EBV/PTLD**
Increasing interest has focused on the prevention of EBV disease and PTLD in organ transplant recipients. Potential strategies for the prevention of EBV disease can be further categorized as immunoprophylaxis, chemoprophylaxis and preemptive therapy.

**Immunoprophylaxis**
Immunoprophylaxis can be categorized as active or passive. Active immunoprophylaxis would be accomplished through the use of an EBV vaccine. Although efforts to develop vaccines have been underway for several years, progress to date has been limited (80–83). Passive immunoprophylaxis is accomplished by providing anti-EBV antibody through the infusion of intravenous immune globulin (IVIG). Published data demonstrated a protective effect of IVIG on the development of EBV disease in a SCID mouse model (84,85) and led to initiation of multicenter, randomized, controlled trial carried out in EBV seronegative pediatric liver transplant recipients. Although statistically significant differences were not observed, the study demonstrated a trend toward decreased rates of EBV disease and PTLD in patients receiving CMV-IVIG compared to those receiving placebo (adjusted 2-year EBV disease free rate, CMV-IVIG 79% versus placebo 71%; PTLD free rate CMV–IVIG 91% vs. placebo 84%) (4). The absence of statistically significant effect of CMV-IVIG in this study may have been due to limitations of sample size, a lack of efficacy of the drug, or the confounding effect of preemptive reductions in immune suppression based upon the presence of an elevated EBV load that occurred in the latter years of this study. Finally, the use of EBV specific cytotoxic T lymphocytes as adoptive immunotherapy could serve as a third potential immunoprophylactic strategy. Unfortunately, although this approach has been proven to be efficacious in stem cell transplant recipients, efforts to translate these benefits to the prevention of EBV disease and PTLD in SOT recipients have not succeeded as of this time (86).

**Chemoprophylaxis**
Chemoprophylaxis using antiviral agents, such as acyclovir and ganciclovir, is one possible approach to the prevention of EBV disease and PTLD. Acyclovir and ganciclovir actively block lytic EBV replication in vitro through...
inhibition of the late phase lytic replication but neither agent has any effect on EBV in its latent state or on the proliferation of EBV-transformed B cells (62,87,88). Accordingly, if progression from EBV infection to disease is dependent upon expansion of EBV immortalized B cells independent of the lytic phase of EBV replication, the use of these agents is unlikely to prevent the development of EBV disease. Unfortunately, only limited evidence is available to address the efficacy of antiviral therapy in the prevention of EBV/PTLD in humans. Published reports support the potential efficacy of antiviral agents have been retrospective and have been limited by the use of either historical or no specific controls (20,89). The difficulty in interpreting the results of such retrospective studies lacking concurrent controls is illustrated by the study by Malouf which reported a drop in the incidence of PTLD from 4.2% to 1.34% after the introduction of ganciclovir prophylaxis in 1996 in lung transplant recipients (90). Unfortunately, the introduction of ganciclovir was coincident with the elimination of antibody for EBV in the study by Opelz et al. found no protective benefit associated with the use of antiviral therapy (14,91). No benefit was found in the only published randomized controlled trial has been completed evaluating the role of antiviral agents in the prevention of EBV/PTLD (92). PTLD developed in 8 of 24 pediatric liver transplant recipients who received 2 weeks of intravenous ganciclovir followed by 50 weeks of high-dose oral acyclovir compared to five cases of PTLD in 24 children who received 2 weeks of intravenous ganciclovir alone (p = NS) (92). Although it is possible that prolonged use of the more potent ganciclovir in lieu of acyclovir might have resulted in a different outcome, development of PTLD in patients while receiving prolonged courses of intravenous ganciclovir has been reported (93).

**Viral load monitoring and preemptive strategies of prevention**

Surveillance monitoring of EBV loads to inform preemptive reductions in immune suppression has resulted in a decreased incidence of EBV disease and PTLD compared to historical controls. McDiarmid and colleagues reported a decreased incidence of PTLD from 10% to 5% using EB viral load monitoring to guide the combined use of reduced immune suppression and intravenous ganciclovir in pediatric liver transplant recipients with rising EBV loads (56). Using decreased immunosuppression alone without ganciclovir in response to elevated EBV loads, Lee and colleagues noted a decline in the incidence of PTLD from 16% to 2% in a group of pediatric liver transplant recipients when compared to historical controls (94). Ganschow and colleagues also showed that lowering immunosuppression in response to results of aggressive viral load monitoring was also associated with low rates of PTLD in pediatric kidney transplant recipients (0.9%) (95).

More recently, Martin et al. explored the use of EBV load monitoring to inform the pre-emptive use of the anti-CD20 monoclonal antibody rituximab in EBV donor positive/recipient negative adult kidney transplant recipients (96). EBV load monitoring was carried out during the first 6 months posttransplant; 33 of 34 patients included in this study received oral valganciclovir for a minimum of 3 months after their transplant procedure. Of interest, the initial detection of 11 of 20 adults with measurable loads occurred while still on valganciclovir. Immunosuppression was reduced in all 20 subjects with detectable loads and PET scans were performed in an effort to identify occult PTLD. Six subjects with persistent elevations for at least two measurements and/or clinical symptoms received one (n = 5) or two doses (n = 1) of rituximab with resultant clearance of their EBV loads. Of note, most of these patients had clinical symptoms at the time that rituximab was given. None of these six developed PTLD. One of the remaining monitored subjects developed CNS PTLD 1 month after clearance of EBV load in the peripheral blood. In contrast, three of six EBV mismatched patients not participating in the monitoring trial developed EBV-associated PTLD during the same time period. Although these results suggest an efficacious effect of this strategy, given its small size, relatively short period of observation prior to use of rituximab and the concomitant reduction of immune suppression, additional experience is needed to confirm these results and determine whether rituximab should only be used in the presence of clinical symptoms versus asymptomatic elevations of load as well as the relative impact of rituximab versus the reduction of immune suppression. Finally, the long-term safety of this approach and efficacy in higher risk pediatric patients remains to be determined.

Based upon available data, it appears that the strategy of using EBV load monitoring to inform preemptive reduction in immune suppression to prevent EBV disease and PTLD is the optimal currently available preventive strategy while more data evaluating the comparative safety and efficacy of rituximab with reduced immune suppression alone in response to rising or elevated EBV loads are needed.

**Conclusions**

Patient outcomes after SOT continue to improve as advances continue in the monitoring, detection and therapy of EBV infection. These improvements have led to a continuing reduction in the incidence of PTLD, as well as greater success in treatment of established disease. The use of EBV viral load monitoring has become the foundation of this success, although the need for international standards to enhance interlaboratory reliability continues. Innovations in monitoring of specific immune function offer an
opportunity for further reduction in PTLD. The combination of cellular immune function monitoring, whether global or EBV-specific, with EBV loads should allow for the discrimination between viral-load positive patients who are at high or low risk of progression to PTLD. Early diagnosis of PTLD facilitates therapy, primarily with reduced immunosuppression, the most effective treatment for disease. The use of anti-CD20 monoclonal antibodies now offers secondary options for therapy, while chemotherapeutic strategies for advanced stage or resistant PTLD continue to improve. The optimal monitoring, prevention and treatment of EBV-associated PTLD remains a dynamic area for investigation and improvement of patient and graft outcomes after SOT.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the American Journal of Transplantation.

EBV Boxes

Box 1: Treatment of EBV and PTLD

- Reduction or cessation of immune suppression is the first line approach to management of EBV/PTLD. Use of this strategy, which is based on the recovery of the host’s immune system and subsequent development of cytotoxic T cell response against EBV, results in a clinical response in as many as 67% of patients. A trial of this approach is generally recommended unless there is concurrent rejection or histological evidence of true malignancy.
- The majority of patients who respond to reduction in immune suppression demonstrate some evidence of clinical response within 2–4 weeks of reduction of immune suppression, though a belated response has been observed.
- Though widely used, the role of antiviral agents such as acyclovir and ganciclovir have not been proven to be efficacious in the treatment of EBV/PTLD. Although both agents inhibit lytic EBV replication in vitro, neither agent has in vitro activity against EBV latently infected B cells, nor have they been effective in treating healthy individuals with acute EBV infection. The vast majority of EBV infected cells within PTLD lesions have been shown to be transformed B cells that are not undergoing lytic infection.
- While a potential rationale exists for the use of IVIG in the treatment of EBV disease and PTLD, definitive evidence in support of the use of this agent is not available. Despite this, some centers will use this in combination with reduction of immune suppression with or without antiviral therapy.
- The anti-CD20 monoclonal antibody rituximab has been increasingly used in the treatment of EBV/PTLD. Increasing published experience from registry reports and case series has been promising leading to a general acceptance of this agent as second line therapy for the treatment of PTLD. An overall response rate of 65% in SOT recipients associated with a long-term cure at a median follow-up of 8 months has been reported. However, relapsed PTLD develops in approximately 20% of responders a median of 7 months after completing their therapeutic course of rituximab which correlates to the time that the biologic effect of this agent is no longer active.
- Several important questions regarding the use of rituximab remain unanswered at this time. These include an incomplete description of the side effect profile of this agent in solid organ transplant recipient including concerns for the potential development of hypogammaglobulinemia and/or additional opportunistic infections in patients treated for PTLD with rituximab. A second important question is the optimal timing of use. While some centers have opted to use rituximab in all patients without an initial period of observation on reduced immunosuppression, the efficacy of this strategy is unknown and unproven. Initial use of the agent without providing the immune system a chance to recover and initiate an immune response to EBV might lead to an increased likelihood of relapse compared to those receiving the agent after failing to respond to reduction of immune suppression.
- The use of low-dose cyclophosphamide/prednisone regimen (used alone or in combination with rituximab) has been evaluated as second line therapy for patients who fail to respond to initial reduction or withdrawal of immune suppression with results demonstrating a 67% 2-year failure-free (without PTLD and with functioning original allograft) survival in children on protocol. Children presenting with fulminant PTLD did poorly with cyclophosphamide/prednisone alone though a subsequent study demonstrated that the addition of rituximab improved outcome for patients presenting with fulminant disease.
- The need for traditional chemotherapy appears to be more frequent in adult compared to pediatric organ transplant recipients with PTLD. While definitive data defining the optimum chemotherapeutic regimen are not available, the most common strategy uses a standard dose cyclophosphamide and prednisone regimen based on treatment of adult B cell lymphomas, and achieves success in approximately two-thirds of patients. More recently, the addition of rituximab to chemotherapy protocols is being explored. While the optimal chemotherapeutic regimen remains to be determined, it is clear that salvage chemotherapy offers a viable option for advanced stages of PTLD.
Box 2: Prevention

- Potential strategies for the prevention of EBV disease can be categorized as immunoprophylaxis, chemoprophylaxis and preemptive therapy.
- Immunoprophylaxis can be categorized as active or passive. Immunization against EBV would establish active immunoprophylaxis, but efforts thus far to develop an EBV vaccine have not been successful. Passive immunoprophylaxis is accomplished by providing anti-EBV antibody through the infusion of intravenous immune globulin (IVIG). Though published data has demonstrated a protective effect of IVIG on the development of EBV disease in a SCID mouse model, the only published randomized trial evaluating this strategy did not demonstrate a statistically significant benefit.
- The role of chemoprophylaxis using antiviral agents, such as acyclovir and ganciclovir, remains unproven and the lone published prospective, randomized trial evaluating this approach failed to demonstrate efficacy. Retrospective studies supporting this approach suffer from methodologic flaws and analysis based upon review of registry data generated conflicting reports.
- The use of surveillance monitoring of EBV loads to inform preemptive reductions in immune suppression has resulted in a decreased incidence of EBV disease and PTLD compared to historical controls and currently appears to be the optimal strategy for prevention of these complications. While some centers have added the use of antiviral therapy to reduction of immune suppression, additive benefit of this approach remains unproven.
- The use of EBV load monitoring to inform the preemptive use of the anti-CD20 monoclonal antibody rituximab in EBV donor positive/recipient negative adult kidney transplant recipients has also been reported with apparent success. Rituximab was used for patients with persistent detectable loads on two serial measurements despite reduction of immunosuppression. Additional experience is needed to confirm the positive results from this single small study as well as to define potential side effects associated with the use of this strategy.

References


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Questions

1. Which of the following would be at the highest risk of developing EBV-associated posttransplant lymphoproliferative disorder following organ transplantation?

   a. A 52-year-old male kidney transplant recipient who was EBV seropositive prior to transplant
   b. An 11-year-old female heart transplant recipient who was EBV seropositive prior to transplant
   c. A 2-year-old liver male liver transplant recipient who was EBV seronegative
   d. A 2-year-old female intestinal transplant recipient who was EBV seronegative prior to transplant

2. Based upon current recommendations and evidence, which of these is the most effective strategy for the prevention of EBV disease and posttransplant lymphoproliferative disorder following organ transplantation in an EBV seronegative recipient?

   a. Vaccination with EBV vaccine prior to transplant
   b. Use of serial doses IVIG after organ transplant
   c. Serial measurements of EBV viral loads in the peripheral blood to inform preemptive reductions in immune suppression
   d. Use of acyclovir or ganciclovir alone as chemoprophylaxis following organ transplantation

3. Diagnostically, which of the following is superior for monitoring for EBV disease after solid organ transplantation?

   a. Serial monitoring of EBV IgG levels in the blood
   b. Serial monitoring of EBV IgM levels in the blood
   c. Serial monitoring of quantitative amount of EBV virus in the blood
   d. Serial monitoring of Alanine aminotransferase (ALT)
   e. Serial monitoring of EBV shedding in saliva

4. A child who was EBV seronegative prior to heart transplant receives a heart from an EBV seropositive donor. Four months after transplantation the child develops high fevers, shortness of breath and was found to have a mass on chest radiograph. Serial EBV viral load testing of the blood demonstrated him to be negative until 2 months of transplant when he had low levels. Today he now has levels which are moderately high in your EBV reference laboratory.

   Which of the following is the most appropriate course of action to perform next?

   a. Computed tomography of the chest, abdomen and pelvis; biopsies of the heart as well as the most accessible lesions suspected of being PTLD followed by decreasing calcineurin inhibitor
   b. Computed tomography of the chest, abdomen and pelvis followed by stopping calcineurin inhibitor
   c. Decrease calcineurin inhibitor and follow serial EBV PCR
   d. Administer CMV hyperimmune globulin and valacyclovir