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Special Article

Epstein-Barr Virus and Posttransplant Lymphoproliferative Disorder in Solid Organ Transplantation

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Abbreviations: ACVBP chemotherapy, (doxorubicin, cyclophosphamide, vindesine, bleomycin, prednisone); ANZDATA, Australia and New Zealand Dialysis and Transplant Registry; ATP, adenosine triphosphate; BAL, bronchoalveolar lavage; CHOP, Cyclophosphamide, Hydroxydaunorubicin (also called doxorubicin or Adriamycin), Oncovin (vincristine), Prednisone or prednisolone; CMV, cytomegalovirus; CNS, central nervous system; CT, computerized tomography; CTL, cytotoxic T lymphocyte; EBV, Epstein-Barr virus; ECOG, Eastern Cooperative Oncology Group; HHV6, human herpesvirus type 6; HIV, human immunodeficiency virus; IL6, interleukin 6; IVIG, intravenous immune globulin; LDH, lactate dehydrogenase; PET, positron emission tomography; PNCL, primary central nervous system lymphoma; PTLD, posttransplant lymphoproliferative disorder; RSST, risk stratified sequential treatment; SRTR, Scientific Registry of Transplant Recipients.

Introduction

Posttransplant lymphoproliferative disorder (PTLD) is recognized as potentially one of the most devastating complications of organ transplantation. The Epstein–Barr virus (EBV) genome is found in the majority (>90%) of B cell PTLD occurring early (within the first year) after solid organ transplantation The entity referred to as EBV-associated PTLD encompasses a wide spectrum of clinical conditions characterized by lymphoproliferation after transplantation, which may or may not be symptomatic. These syndromes range from uncomplicated infectious mononucleosis to true malignancies (1–3). Disease may be nodal or extranodal, localized, often in the allograft, or widely disseminated. PTLD may resemble a self-limited infection or be indistinguishable from non-Hodgkin's lymphoma. Lesions may be localized and progress slowly or the patient may present with a fulminant multisystem sepsis-like syndrome.

EBV is known to play a major role in the development of PTLD (4). The pathogenesis of these disorders is complex, and related to EBV's ability to transform and immortalize B lymphocytes, sometimes combined with secondary genetic or epigenetic events that occur during uncontrolled proliferation. Host and viral genomics affecting the response to EBV infection, local environmental factors including chronic antigenic stimulation, and the presence of other infections may impact outcome. Immunomodulation caused directly by EBV viral proteins, the coordinated effects of viral and cellular miRNAs (5) and exogenous immunosuppressive drugs alter the proliferative response and survival of infected cells (6,7) and the innate and adaptive immune responses, particularly the EBV-specific cytotoxic T lymphocyte (CTL) responses critical for controlling EBV infection.

Although B cell transformation and PTLD are a result of latent EBV infection, lytic EBV infection appears to be extremely important during primary EBV infection prior to the development of the CTL response (8). For a patient experiencing EBV infection for the first time in the early posttransplant period, delay in development of the immune response theoretically would prolong the one-way self-amplifying circuit of naïve B cell infection, latency in memory cells and reactivation with infectious virus production. The resulting high virion peak results in massive infection of the B cell pool and perhaps other cells not normally infected (T cells, NK cells, memory B cells), thereby setting the stage for secondary events that lead to malignancy. Although the role of EBV in EBV-negative PTLD is uncertain, recent data support the hypothesis that over time, immune escape occurs in initially EBV-driven lymphoproliferation, with cellular mutations replacing the functions of EBV oncogenes (9).

This document summarizes current recommendations and supporting data that guide the prevention, diagnosis and treatment of PTLD in the solid organ transplant recipient. The recent literature was reviewed, including recommendations for the diagnosis and management of PTLD that were published by notable groups (e.g. the British Transplantation Society [10,11]). Although the focus is largely on PTLD, relevant aspects of non-PTLD EBV syndromes are addressed, as appropriate.

Epidemiology

Humans are the only known hosts of EBV. In immunocompetent individuals, this virus is transmitted in the community by exposure to infected body fluids such as saliva. Although infection may also be acquired in the community by the traditional routes of transmission seen in immunocompetent patients, for solid organ transplant recipients, EBV that is transmitted from the seropositive donor organ is an important source of infection. Transmission is also possible when nonleukoreduced blood products are used. In the least affluent nations, greater than 90% of individuals are EBV-seropositive before the age of 5 years (12). However, in more affluent developed nations, this level of seropositivity is not attained until the fourth decade of life.

The diagnosis of PTLD requires tissue examination. In many settings tissue is not available or accessible. When laboratory evidence of EBV infection is present and other causes have been ruled out, investigators have used the term EBV "disease" to describe a number of clinical syndromes where EBV is believed to play a causative role.

Although the highest rate of PTLD in the solid organ transplant setting is seen in the first year after transplant, recent analyses suggest that the incidence of early PTLD is decreasing (13,14). However, cases occurring in the first year after transplant represent only one-fifth of the total cumulative 10-year post transplant PTLD burden (15). Analyses of both French and ANZDATA renal PTLD registries suggest a biphasic pattern of disease with a second peak occurring in years 7–10 after transplant after a period of reduced incidence in years 2–7. A significant proportion of late B cell PTLD is monomorphic and may be EBV-negative (~20%), with the relative proportion of EBV-negative lesions increasing over time after transplant; NK or T cell PLTD (approximately 37% are EBV positive) may also occur late after transplant (16). As transplant patient survival improves, late and EBV-negative PTLD will represent an increasing proportion of cases seen in adult populations. Although historically the median time of onset of primary EBV infection after solid organ transplantation is 6 weeks and reactivation/infection events were most often observed in the 2–3-month period after transplantation, recent studies in patients monitored serially using EBV viral load, note later initial detection of EBV DNAemia at a median of 110 days (17) and a mean of 276 days (18). PTLD incidence is also dependent on the type of organ transplanted, which may reflect immunosuppressive regimens, lymphoid load in the allograft and chronic antigenic exposure when organs directly communicate with the environment (8). Small intestine transplant recipients are at the highest risk for development of PTLD (up to 32%), while recipients of pancreas, heart, lung and liver transplants are at moderate risk (3–12%). Renal transplant recipients are at relatively low risk (1–2%). Recently, Caillard also described a temporal sequence of sites of PTLD involvement in adult renal allograft recipients, with disease localized to the graft occurring within the first two years, CNS disease occurring between years 2 and 7 and gastrointestinal disease occurring between years 6 and 10 and becoming the predominant site of late disease (13). Although PTLD in solid organ transplant recipients is most often of recipient origin (19), PTLD limited to the graft occurring early after transplant is predominantly donor in origin (20).

Table 1: Risk Factors for PTLD in solid organ transplant recipients

Early PTLD
Primary EBV infection
Type of organ transplanted
OKT3 and polyclonal antilymphocyte antibodies
Young recipient age (i.e. infants and young children)
CMV mismatch or CMV disease
Late PTLD
Duration of immunosuppression
Type of organ transplanted
Older recipient age (i.e. adults)

Contradictory/controversial evidence exists for the role of the following as risk factors for primary disease: Tacrolimus in pediatric recipients; HLA matching; certain cytokine gene polymorphisms; preexisting chronic immune stimulation; Hepatitis C infection; viral strain virulence (EBV1 vs. EBV-2 and LMP1 deletion mutants).

Risk Factors

The risk factors for the development of early (<12 months after transplant) and late PTLD (>12 months after transplant) in solid organ transplant recipients are shown in Table 1 (21–24). Analyses of risk factors for PTLD have used both smaller single center and larger registry datasets. Both approaches have limitations and often involve specific subsets of patients, adults versus children or specific allograft types. Many of the risk factors are interrelated and multivariate analysis is required to identify independent risk factors. Even using this approach, results are not always consistent (25). An overwhelming risk factor in most analyses is primary EBV infection, placing pediatric populations at higher risk of developing PTLD than their adult counterparts (14,26). Surprisingly, in a recent Collaborative Transplant Study database analysis, pretransplant EBV seronegativity in liver transplant recipients, unlike other allograft types, was not associated with an increased risk of developing non-Hodgkin's lymphoma. However, a subsequent analysis of the SRTR data in the United States confirmed that being EBV seronegative was a risk

factor for PTLD development even in liver transplant recipients (but less so than in kidney and heart transplant recipients) because of a higher baseline risk in seropositive liver transplant recipients (27). Individuals who are R+ are not devoid of PTLD risk, and account for up to 25% of PTLD cases in children (28). Intestinal transplant recipients who are EBV-seropositive remain at a high risk of PTLD. Although, PTLD rates increased after calcineurin inhibitors became the backbone of most immunosuppressive regimens in the 1990s, it is likely that the net state of immunosuppression, an entity difficult to measure, is a major risk factor. Attempts to quantify the risk associated with specific immunosuppressive agents used for induction or maintenance therapy have often led to inconsistent results (25,29). Antilymphocyte globulins that result in selective T cell depletion, particularly when used in high dose or repetitive courses, have historically been associated with increased PTLD risk. Among the newer biologic agents, alemtuzumab does not seem to be associated with an increased PTLD risk. Very high rates of PTLD presenting predominantly as primary CNS lymphoma were observed in renal transplant patients who received belatacept and were EBV seronegative prior to transplant, leading to prohibition of the use of this agent in this subset of patients (30–32). The duration of immunosuppression and older recipient age are risk factors for late PTLD development. This highlights the need for studies to optimize minimization of long term immunosuppression in individual patients including the accommodation of immunosenescence associated with aging in patients surviving for long periods after transplant. Cytomegalovirus infection may contribute to the net state of immunosuppression and is known to be a risk factor for PTLD.

Manifestations of Non-PTLD EBV Syndromes

Although the most feared EBV-associated disease after transplantation is PTLD, patients may experience non-PTLD-related disease. The features of this might include the manifestations of infectious mononucleosis (fever, malaise, exudative pharyngitis, lymphadenopathy, hepatosplenomegaly and atypical lymphocytosis), specific organ diseases such as hepatitis, pneumonitis, gastrointestinal symptoms and hematological manifestations such as leucopenia, thrombocytopenia, hemolytic anemia and hemophagocytosis. Some of these manifestations may be identical to the features of PTLD (Table 2). EBV-associated posttransplant smooth muscle tumors can occur *de novo* or after PTLD at a median interval of 48 months after transplant and develop earlier in children than adults. They can be of donor or recipient origin, and appear in atypical sites such as solid organs. When involving multiple sites, disease is multifocal rather than metastatic in origin (33). HHV6 reactivation may theoretically be an indirect cofactor for PTLD due to the potential for interaction with CMV (34).

Table 2: 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	Mass lesions
Gastrointestinal bleeding	
Symptoms of bowel perforation	

Manifestations and Diagnosis of PTLD

Clinical assessment

Relevant clinical information includes, but is not limited to the following:

- EBV serostatus of transplant recipient and donor.
- CMV donor/recipient serostatus.
- Time from transplantation to PTLD diagnosis.
- Type of allograft.

An adequate physical examination is required to detect the manifestations of PTLD, which may be quite nonspecific (Table 2). Given the predilection for the reticuloendothelial system to be involved, this clinical examination should include a meticulous assessment for lymphadenopathy and adenotonsillar hypertrophy. The general physical examination might elicit signs referable to the site(s) of organs affected by PTLD.

Laboratory tests

Blood tests (Non-EBV): Initial tests include a complete blood count with white blood cell differential. In the case of the latter, lymphopenia might suggest less overall CTL activity, which is essential in containing EBV-driven lymphoproliferation. In some patients with PTLD, there may be evidence of anemia, which is usually normochromic, normocytic, but may be hemolytic. 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, such as examination of the stools for occult blood. Thrombocytopenia has also been observed in non-PTLD EBV disease.

Depending on the location of PTLD lesions, there may be evidence of disturbances 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.

CMV infection status should be determined using CMV pp65 antigenemia assays, plasma or whole blood quantitative nucleic acid testing for CMV DNA as well as the examination of biopsy tissue for viral inclusions, CMV DNA or CMV antigens by immunohistochemistry.

Other adjunctive tests that might predict PTLD risk have been investigated. Promising initial results have been obtained for biomarkers that include serum 1L-6 (35), serum/plasma free light chains (36), serum sCD30 (37), serum CXCL13 (38) and host genetic polymorphisms particularly in cytokine genes (25) but require further validation. How these markers relate to each other and to EBV viral load in predicting PTLD risk should be the subject of future research.

Blood tests (EBV-related)

EBV serology: In immunocompetent patients, primary EBV infection can be determined by measuring EBV antiviral capsid antigen IgM and IgG antibodies, antibodies to early antigen (EA) and Epstein–Barr nuclear antigen. Persistence of anti-EA antibodies has been shown to be more likely in PTLD patients (39) 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 (40). Serology is unreliable as a diagnostic tool for either PTLD or primary EBV infection in immunocompromised patients, due to delayed or absent humoral responses. Another important drawback is that if these patients are receiving blood products, the passive transfer of antibodies may render EBV IgG antibody assays difficult to interpret. The most important role of EBV serology in the setting of transplantation is the determination of pretransplant donor and recipient EBV serostatus for PTLD risk assessment.

Detection of EBV nucleic acids or protein in tissue: Documenting the presence of EBV-specific nucleic acids in tissues is of value in the diagnosis of EBV-associated PTLD. RNA *in situ* hybridization targeting EBV-encoded small nuclear RNA (EBER; Refs.41,42) is the preferred approach and is more sensitive for detecting EBV-infected cells than *in situ* hybridization directly targeting viral DNA because EBERs are expressed at levels several orders of magnitude higher in infected cells. EBV latent or lytic antigens can also be detected in fixed tissues by immunohistochemistry using commercial antibodies directed against EBNA-1, EBNA-2 and LMP-1 or BZLF1, respectively (41,43) and used to document the presence of EBV although these techniques are less sensitive than *in situ* hybridization. Direct EBV DNA amplification from tissue is less useful as it does not allow cellular localization or differentiation of EBV in lesions from that present in passenger lymphocytes.

Viral load determination: The optimal way to perform, interpret and utilize quantitative EBV viral load assays for surveillance, diagnostic and disease monitoring purposes remains uncertain (44). In October 2011, the World Health Organization approved the 1st International Standard for EBV created by the National Institute for Biological Standards and Controls for calibration of the wide array of commercial and in house developed assays currently being used for EBV nucleic acid testing. This international reference standard should reduce the significant and extreme interlaboratory variability in both qualitative and quantitative viral load results previously documented (45,46). Until the impact of the standard on result harmonization among assays is validated, interinstitutional result comparison requires formal crossreferencing of assays between institutions. Data suggest that in most laboratories intralaboratory result reproducibility and result linearity over the dynamic range of the assay is reasonable. Therefore trends in patients over time within individual institutions using a single assay are valid and more useful than single values (45,46). Optimal extraction methods, gene targets and instrument platforms for EBV viral load assessments have not been determined. Although EBV viral load in whole blood and lymphocytes appears comparable and normalization of reporting units to cellular DNA does not change dynamic trending in individual patients (reporting IU/mL of whole blood is adequate), controversy with respect to preferred sample type (whole blood vs. plasma) remains and should be the focus of future research studies (47–49). Whole blood or lymphocyte EBV viral load monitoring is more sensitive than plasma for detection of early EBV reactivation. Although, generally, EBV DNA becomes detectable in plasma as EBV viral load rises in matched whole blood samples, the quantitative correlation between EBV viral load measured in whole blood or lymphocytes versus plasma is suboptimal.

Studies of the sensitivity and specificity of quantitative EBV viral load for the diagnosis of early PTLD and symptomatic EBV infection are limited (50–53). Pediatric populations have been the focus of many of these studies. Data from prospective studies targeting adult patients are limited (54,55). In high-risk asymptomatic solid organ transplant recipients being serially monitored, the use of EBV viral load as a diagnostic test (i.e. levels above a specific quantitative threshold being diagnostic of PTLD) has good sensitivity for detecting EBV-positive PTLD but misses EBV-negative, some cases of localized and donor-derived PTLD. However, it has poor specificity, resulting in good negative (greater than 90%) but poor positive predictive value (as low as 28% and not greater than 65%) in these populations. When used in the diagnostic context, this would result in significant unnecessary investigation of patients for PTLD.

Formal evaluation of EBV viral load assessments as a diagnostic tool using a single evaluation in patients presenting with symptoms and/or signs (usually mass lesions) with no history of recent or previous monitoring have not been carried out in populations at high risk for PTLD. In low-risk seropositive adult transplant recipients presenting for

investigation with signs and symptoms compatible with PTLD, high EBV viral load lacked sensitivity, understandably missing all cases of EBV-negative PTLD and some cases of localized EBV-positive PTLD, but was highly specific for EBV-positive PTLD (52). EBV viral load measured in plasma appears to improve the specificity of the test as a diagnostic tool for EBV-positive PTLD while not significantly lowering its sensitivity relative to assessments in cellular blood compartments (50–53,56). Preliminary data suggest that EBV viral load testing in samples other than peripheral blood, that is, broncoalveolar lavage (BAL) fluid or CSF may be useful. Among pediatric lung and heart lung transplant patients in whom the lung is often the primary site of PTLD, high quantitative levels of EBV load in BAL fluid may be a more sensitive predictor of PTLD than peripheral viral load assays (57). However, EBV DNA, often at high levels were detected in BAL fluid of adult lung transplant recipients in the absence of PTLD (58). Similarly, extrapolating from experience in HIV-infected patients, qualitative and quantitative EBV testing in CSF is performed to assist in the diagnosis of CNS lymphoma (59). However, further data regarding the sensitivity and specificity of testing in BAL and CSF are required in order to meaningfully interpret testing at these sites.

Adjunctive laboratory testing may improve the specificity of high viral load as a predictor of PTLD. The best studied and most promising are assays measuring T cell restoration or EBV-specific T cell responses (60). Although data suggest that the specificity and positive predictive value of EBV viral load can be significantly improved by using concomitant EBV-specific T cell ELISPOT and tetramer assays, these assays are complex, costly and difficult to implement in a routine diagnostic laboratory (10). Simpler rapid assays to measure global and EBV-specific T cell immunity using commercial ATP release assays (Cylex Immuknow and T Cell Memory) have undergone preliminary evaluation as adjunct markers of PTLD risk when combined with viral load testing in pediatric thoracic transplant recipients but require further validation (61). Viral gene expression profiling in peripheral blood as an adjunctive test of PTLD risk has been studied (62) and is still the subject of research. To date no distinctive pattern that is indicative of PTLD or PTLD risk has been demonstrated.

Radiographic imaging: Most centers employ a total body CT scan (head to pelvis) as part of the initial assessment of PTLD. Beyond this, the choice of tests depends largely on the location of suspected lesions and the historical sequence of prior radiographic testing. Many experts recommend that a head CT or MRI be included as part of the initial work-up, as the presence of central nervous system lesions will significantly influence treatment and outcome. 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. Depending on the location (e.g. CNS lesions), MRI may be a more suitable modality than CT scanning due to radiation concerns with CT scans and more precise lesion delineation with MRI.

Pulmonary lesions that are visible on chest radiographs may require high-resolution CT scanning for better delineation prior to biopsy. 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, persistent diarrhea and unexplained weight loss, where necessary.

Positron emission tomography–computerized tomography (PET–CT) is emerging to be a useful test in the evaluation of PTLD (63,64), although additional data are needed on its utility across the known heterogenous spectrum of PTLD lesions. It may be more useful for monitoring response to therapy than for initial diagnosis. A major disadvantage is that the amount of radiation exposure is significantly greater than that associated with regular CT scans.

Histopathology: Pathology remains the gold standard for PTLD diagnosis (2,65). Although excisional biopsy is preferred, needle biopsy is acceptable when larger biopsies are impractical as in the case of allograft organ biopsy. The tissue specimen should be interpreted by a hematopathologist or pathologist familiar with histopathologic features of PTLD. Institutional protocols should be put in place to ensure that tissue is handled appropriately for ancillary diagnostic tests.

It is essential that reactive conditions such as plasma cell hyperplasia and infectious mononucleosis be clearly segregated in the classification process from potentially neoplastic lesions, which contain monoclonal elements. The Society for Hematopathology has published a working categorization of PTLD under the auspices of the World Health Organization (65) and is recommended for use (III). Table 3 summarizes the key features of this classification system. Intrinsic weaknesses are present in the purely histologic classification of PTLD. Additional pathologic tools have provided a better understanding of the pathogenesis of PTLD with the goal of developing more effective and more targeted therapy. Use of ancillary diagnostic tests identified as essential is strongly recommended if available (AIII). In addition to EBER and the detection of latent antigens as outlined previously, these tests are as follows:

- Immunophenotyping to determine lineage and therapy dependent markers (i.e. CD20) (essential).
- EBV clonality studies (rarely required/research).

Table 3: Categories of posttransplant lymphoproliferative disorder (PTLD)

Early lesions[1]
Plasmacytic hyperplasia
Infectious mononucleosis-like lesion
Polymorphic PTLD
Monomorphic PTLD
(classify according to the lymphoma they resemble)
B cell neoplasms
Diffuse large B cell lymphoma
Burkitt lymphoma
Plasma cell myeloma
Plasmacytoma-like lesion
Other[2]
T cell neoplasms
Peripheral T cell lymphoma, NOS
Hepatosplenic T cell lymphoma
Other[2]
Classical Hodgkin lymphoma-type PTLD

[1]Some mass-like lesions in the posttransplant setting may have the morphologic appearance of florid follicular hyperplasia or other marked but non-IM-like lymphoid hyperplasias.
[2]Indolent small B cell lymphomas arising in transplant recipients are not included among the PTLD.

- Molecular genetic markers of antigen receptor genes to assess clonality (useful).
- Donor versus recipient origin (useful).
- Fluorescent *in situ* hybridization or gene profiling by microarray to detect alterations in oncogenes, tumor suppressor genes or chromosomes (rarely required/research).

Recurrent PTLD may represent true recurrences (morphologically and clonally identical to the original tumor), PTLD in a more aggressive form or the emergence of a second primary tumor such as an EBV-associated posttransplant smooth muscle tumor. For this reason, biopsy of such recurrences is encouraged (III) (2).

Clinical staging of PTLD

No staging system currently exists for PTLD and no single system totally captures the full spectrum of what is classified as PTLD. Although the Ann Arbor staging has been used with the Cotswold's modifications, other staging approaches such as the Murphy system have been used in children (66). 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 of CNS involvement. Additional investigations such as a bone scans, a bone marrow biopsy and a lumbar puncture may assist in ruling out bone, bone marrow and CNS disease, respectively. In cases of EBV-positive PTLD documented by immunohistochemistry or *in situ* hybridization, an EBV viral load assay should be performed in order to better document the incidence and natural history of EBV viral load negative but EBV positive PTLD cases.

Prevention of PTLD

Although some centers employ chemoprophylaxis and/or preemptive strategies using EBV viral load as a surveillance tool, for the prevention of this complication, published data in the form of prospective controlled trials in support of these protocols are currently limited and the role of antiviral agents is controversial. Potential strategies for prevention are listed below.

General

Identification of patients who are also at risk of primary CMV infection or severe CMV disease or receiving antithymocyte globulin for induction or rejection would select a particularly vulnerable subgroup of recipients since these factors have been identified as risk factors for PTLD. Such patients should be monitored carefully for clinical symptoms/signs (fever, diarrhea, lymphadenopathy, allograft dysfunction, etc.) and investigated aggressively for PTLD. Allograft biopsies from these patients should be reviewed carefully for evidence of early PTLD. Wherever appropriate, immunosuppression should be minimized and aggressive immunosuppression should only be employed in the presence of biopsy proven acute rejection (65) (II-2). Because PTLD frequently presents with allograft dysfunction, it is important to make a pathologic diagnosis of rejection using standardized criteria and clearly distinguish early PTLD from rejection prior to the use of more potent antirejection therapy. The use of techniques to identify EBV-infected cells in tissues would be useful in this setting.

Antiviral prophylaxis

Chemoprophylaxis: Some centers have adopted antiviral prophylaxis as standard of care for high-risk patients (EBV D+R–). Although the antiviral agents, acyclovir and ganciclovir, have been employed as prophylaxis for the prevention of PTLD, data to support this are limited and a definitive recommendation regarding their use cannot be made at this time (I). Because CMV disease is a cofactor in PTLD development, if employed, the use of ganciclovir is preferable to acyclovir use (67). However, PTLD has been documented in patients receiving antiviral prophylaxis. Although a case-control study in renal transplant recipients suggest antiviral therapy may reduce PTLD risk (II-2) (67), analysis of the Collaborative Transplant Study database suggested that the use of antiviral drugs does not reduce the risk of posttransplant lymphoma (70). EBV load has been shown to progressively rise in some patients while patients were on ganciclovir prophylaxis (68). The impact of antiviral drugs on lytic virus could potentially decrease the recruitment of newly infected cells and the subsequent generation of latently infected memory cells, leading to a long term decrease in viral load measured in cellular blood compartments; these responses might not be readily apparent in the short term as assessed by EBV viral load monitoring. Antiviral therapy may have an indirect benefit on PTLD development by eliminating other viral infections which act

as cofactors in the lymphoproliferative process (III). However, these theoretical considerations remain unproven and there is currently no definitive evidence that such antiviral effects would be beneficial in preventing PTLD.

Immunoprophylaxis: Prospective randomized trials of CMV–IVIG, and ganciclovir plus CMV–IGIV, respectively have been inconclusive (68,69). An epidemiologic study by the Collaborative Transplant Group found that the use of anti-CMV IVIG reduced the incidence of non-Hodgkin lymphoma in kidney transplant recipients but only in the first posttransplant year (70). Thus, although prophylaxis with immune globulin may have some effect in reducing the short-term risk of PTLD, data are limited. At this time an all-encompassing recommendation of the utility of this approach cannot be made (I). Preventing EBV infection by vaccination is currently the subject of research (71). A phase I/II study indicated transient humoral immune response to an EBV recombinant gp350/alhydrogel vaccine among children with chronic kidney disease (potential transplant candidates; Ref.72).

Preemptive management

Since high viral load states often antedate the clinical presentation of PTLD, there are data to support quantitative EBV viral load monitoring for PTLD prevention in high-risk populations (50,53). Data to support this approach in populations at low risk of PTLD such as adult transplant recipients seropositive for EBV before transplant are lacking. Optimal monitoring frequency is uncertain. Since EBV viral load doubling times as short as 49–56 h have been documented, frequent (weekly) monitoring over the high-risk period has been recommended by some investigators. However, there are no data to suggest that less frequent monitoring (i.e. biweekly or at even longer intervals later in the first year after transplant) negatively impacts preemptive management. Weekly to biweekly monitoring over the first year after transplant is recommended, although this may be logistically difficult to implement over the entire period (II-3). There are insufficient data to support routine monitoring beyond the first transplant year. Data regarding the natural history of EBV viral load in transplant recipients in the absence of intervention are limited. This, along with lack of assay harmonization, prevents clear definition of "trigger points" that can be applied across all organ types that are predictive of PTLD development and at which preemptive intervention should take place.

Preemptive strategies in the solid organ transplant setting most commonly involve the use of reduction of immunosuppression and antiviral agents ± immune globulin (73) or the reduction of immunosuppression as the sole strategy (74). Some centers have reported a reduction in incidence of PTLD when routine viral load monitoring and these preemptive strategies were applied compared to historical cohorts (II-2). A retrospective study of EBV adult mismatched renal transplant recipients suggested that preemptive rituximab may have had an impact on PTLD development (75). The absence of a control group and the inability to differentiate between rituximab and the influence of viral load monitoring itself on immunosuppression management in this study precludes any firm conclusions regarding the efficacy of preemptive rituximab. More aggressive interventions involving the use of low dose rituximab (76) and adoptive immunotherapy (77) have been studied primarily in hematopoietic stem cell transplant recipients; some measure of success has been observed. Data regarding adoptive immunotherapy use in the solid organ transplant setting are more limited; proven efficacy remains uncertain (78,79) (II-3). Reduction in immunosuppression remains the best-validated preemptive strategy. Currently, there is insufficient evidence to recommend the use of either preemptive rituximab or adoptive immunotherapy for preemptive management (III).

Treatment of PTLD

The treatment of PTLD remains a challenge. Currently, there is no unifying consensus that dictates the specific treatment approaches that should be undertaken for all categories of patients. Controlled interventional studies are lacking. The general approach to therapy involves a stepwise strategy that starts with reduced immunosuppression, with plans for further escalation of treatment based largely on the clinical response and the histopathologic characteristics of the PTLD. Due to the highly specialized nature of the diagnosis, staging and treatment of PTLD, the initial evaluation and management of such patients should be done by or under the supervision of a tertiary transplant center and involve a multidisciplinary team that includes transplant physicians, oncologists and infectious disease specialists.

Reduction of immunosuppression

Over the past 25 years, reduction in immunosuppression has been a common initial approach to PTLD management, but reported response rates have been highly variable (0–73%), likely reflecting the heterogeneity and size of the populations studied and the nonstandardization of immunosuppression reduction. Among the largest studies examining this issue is a recent single center report that retrospectively analyzed outcomes in 67 adult solid organ transplant PTLD patients managed with a standardized approach to immunosuppression reduction alone as initial therapy (80). An overall response rate of 45% (37% complete response) was observed; patients who achieved complete remission had relapse rates of 17%. Although neither EBV-seronegativity nor B cell histologic subtype influenced outcome, bulky disease, advanced stage and older age predicted lack of response. Of concern were the high rates of acute rejection (32%) observed. It is unclear whether these data are applicable to pediatric populations who are more likely to experience PTLD in the context of primary infection. In patients who do not have rapidly

progressive disease and who lack predictors of poor response to immunosuppression reduction, reduction of immunosuppression to the lowest tolerated level is recommended as initial therapy for early and late B cell PTLD (II-3). The optimal strategy for immunosuppression reduction is uncertain and may be allograft specific, depending on the comfort of the physicians in risking acute rejection events. Suggestions for reducing immunosuppression based on expert opinion are outlined in the British Transplantation Society PTLD management guidelines (10). The period one should wait before proceeding to alternative therapeutic interventions is also uncertain. Most patients would be expected to show evidence of a clinical response to reduced immunosuppression within 2–4 weeks (81) but since the median time to failure in nonresponders was 45 days in the study by Reshef et al. (80), waiting up to 6 weeks in stable patients without evidence of progressive disease could be considered (II-3).

Surgical resection/local irradiation

Complete or partial surgical resection, as well as local radiotherapy, have been used as adjunctive therapy along with reduced immunosuppression (82). When surgical excision or radiotherapy has been used for localized disease, long-term remission in the absence of additional therapy has been observed (81,83). Surgery is an essential component of the management of local complications such as gastrointestinal hemorrhage or perforation (III).

Antiviral agents (acyclovir, ganciclovir)/passive antibody (IVIG)

Acyclovir and ganciclovir have been used in the management of early PTLD, alone or in combination with immune globulin (1,3,28). Currently, when antiviral agents are employed, the agent of choice is ganciclovir, as *in vitro* it is 10 times more active against EBV compared with acyclovir. The efficacy of this approach is uncertain and there is no evidence to support the use of antiviral agents in the absence of other interventions such as decreasing immunosuppression or anti-CD20 therapy (III). Arginine butyrate, a histone deacetylase inhibitor induces the lytic cycle of EBV, making EBV-infected cells sensitive to ganciclovir. A phase I/II trial of arginine butyrate combined with ganciclovir demonstrated overall response rates in 10 of 15 patients with EBV+ lymphoid malignancies; one third had PTLD (84). Unfortunately this agent is no longer available for use in clinical settings. Another chemotherapeutic agent, the proteosome inhibitor bortezomib, also induces lytic virus replication in EBV infected cells and is currently being evaluated in clinic trials of gamma-herpesvirus associated malignancies including PTLD (85).

Monoclonal B cell antibody therapy (Anti-CD20)

Although single agent rituximab, an anti-CD20 humanized chimeric monoclonal antibody, is rarely effective in the treatment of high grade B cell lymphomas in the immunocompetent patient, complete and sustained responses have been observed using this treatment approach in PTLD. Three prospective phase II rituximab monotherapy trials demonstrated a combined overall response rate of 55% (86) and in a large retrospective review early rituximab therapy improved progression free and overall survival (87). Gonzalez-Barca (88) reported complete response rates improving from 34.2% to 60.5% with a further four doses of rituximab in patients who achieved partial remission with the initial four doses. Although treatment is well tolerated, relapse is not infrequent after four courses of rituximab, with 25% of patients who had partial or complete responses showing evidence of disease progression by one year after treatment in one study (89). There is limited evidence to suggest that relapsed patients can be successfully retreated with single agent rituximab (90). Choquet proposed a prognostic score composed of age >60, Eastern Cooperative Oncology Group prognostic index of 2–4 and raised LDH that predicted survival after rituximab monotherapy and suggested that patients with one or more of these risk factors would benefit from rituixmab in combination with chemotherapy as initial therapy. In a prospective PTLD treatment trial of 4 weeks of rituximab therapy followed by four sequential cycles of rituximab/CHOP every 3 weeks (cyclophosphamide, doxorubicin, oncovin and prednisone) called sequential therapy, interim analysis suggested that response to the first 4 weeks of rituximab correlated with survival (86). An approach known as risk-stratified sequential therapy (RSST) is an alternate more tailored approach, whereby patients who achieved complete remission with an initial four doses of rituximab received a second course of rituximab without chemotherapy. Optimal number and timing of doses is unclear when this rituximab monotherapy approach is used. British guidelines suggest 8 weeks of rituximab (10); the future RSST trial proposed will use four additional courses or rituximab at three weekly intervals in patients who achieve complete remission after four initial weekly courses of rituximab (86). There is a growing body of evidence in support of the use of rituximab as the next step in the treatment of CD20+ B cell PTLD after reduction in immunosuppression in low risk patients who lack risk factors outlined by Choquet above (II-1). Potential adverse events include a tumor-lysis like syndrome, prolonged depletion of B cells with protracted hyprogammaglobulinemia, intestinal perforation, CMV reactivation, and progressive multifocal leukoencephalopathy. Although experience with the use of this agent is increasing, there is an ongoing need for data from prospective clinical trials.

Cytotoxic chemotherapy

In studies usually retrospective and involving a relatively small number of patients, cytotoxic combination chemotherapy, usually CHOP but also ACVBP (doxorubicin, cyclophosphamide, vindesine, bleomycin and prednisone) and ProMACE CytoBOM (mechlorethamine, doxorubicin, cyclophosphamide, etoposide, vincristine, prednisone, procarbazine, methotrexate, cytarabine, bleomycin) has been used to treat PTLD. Complete remission rates varying from 42–92% (87). Although this approach offers

better long-term disease control than rituximab monotherapy, treatment related mortality is high at 13–50%, usually from infectious complications. Outcomes in the largest prospective PTLD treatment trial, in which sequential treatment with rituximab and CHOP as described above was used in 74 adult patients with ECOG >2, have recently been reported (86). The overall response rate 90%, complete response rates 68%, and median response duration was >79.1 months in the 53 patients who responded. This was better than the response of rituximab monotherapy followed by chemotherapy at relapse, and the authors argue that this approach should be applied to all patients not responding to immunosupression reduction. However, CHOP associated treatment-related mortality at 11% predominantly related to infection was observed, suggesting that a more tailored approach that identifies patients who may sustained responses to rituximab monotherapy alone and avoids the toxicity of chemotherapy might be preferred. In pediatric populations, multicenter prospective studies using six cycles of low dose cyclosphosphamide and prednisone with and without rituximab after failure of initial therapy, most often reduction of immunosuppression have been reported (91,92). Response rates (67%, 69%) and relapse rates (19%, 8%) without and with rituximab, respectively were observed. Addition of rituximab therapy appeared to add efficacy to the management of fulminant disease which was not responsive to low dose chemotherapy alone. The use of chemotherapy should be considered after failure of reduction in immunosuppression in adults who have risk factors predicting poor response to rituximab monotherapy, patients who fail to achieve complete remission after initial rituximab therapy (II-1), and in the setting of T cell, Burkitt or Hodgkin lymphoma (III).

Other treatment modalities

Adoptive immunotherapy: Adoptive immunotherapy using donor derived cloned EBV-specific cytotoxic T cells has been used successfully for both the prevention and treatment of PTLD in allogeneic stem cell transplant recipients (76), but in the solid organ transplant setting experience is limited. Obstacles include the fact that PTLD lesions are usually of recipient origin in contrast to donor origin in the stem cell transplant recipient. Cost and time required to clone cell lines may also limit the utility of this approach. Although dramatic and sustained responses (52%) of PTLD, including CNS PTLD, that had failed conventional therapy including chemotherapy and rituximab, have been observed using HLA-matched unrelated donor EBV–CTL in a prospective multicenter trial, these biologic products are currently not readily available (93). Thus, additional research is needed to define the role of adoptive immunotherapy in the solid organ transplant setting and create the infrastructure, which might produce and distribute such products.

Immunomodulatory/Anticytokine therapy: Alpha interferon has both antiviral and antiproliferative activity, and additionally affects the host immune response via its activity as a T helper type 1-associated cytokine. Limited data in solid organ transplant recipients indicate that some patients may respond to alpha interferon in conjunction with a reduction in immunosuppression (94) (III). However, there are concerns that interferon therapy could precipitate rejection. Thus, this agent is no longer commonly employed in the treatment of PTLD and its place in the stepwise management of PTLD has been largely replaced by anti-CD20 monoclonal antibody. Anti-IL6 therapy has been explored in the treatment of early PTLD (95). Data are limited and additional research is needed.

CNS disease: Because CNS PTLD is a rare disease, clinical trial data and standardized management approaches that might inform optimal treatment approaches are lacking. Current recommendations that include the use of whole brain irradiation or high dose methotrexate as first line therapy rely heavily on the experience in immunocompetent patients with primary CNS lymphoma (PCNSL) (10,87,96). However, the former approach is associated with significant neurotoxicity particularly in older patients and when the latter approach is used, renal and hepatotoxicity can be difficult to manage in a transplant setting. The inability of rituximab to cross the blood–brain barrier has raised concerns that levels achieved with systemic use alone are unlikely to have clinical efficacy in CNS PTLD. However, Cavaliere (97) observed surprisingly good outcomes in seven of eight SOT recipients with PCNSL treated with primary rituximab monotherapy, often with reduction in immunosuppression in the absence or either chemotherapy or radiotherapy. Over the past decade there has been an increasing number of additional case reports in transplant recipients with PCNSL achieving complete remission using either standard or escalating doses of rituximab alone (98). Although high dose methotrexate or local radiotherapy should be considered as treatment options in patients with CNS disease who are able to tolerate therapy (II-3), in stable patients systemic rituximab therapy and initial reduction in immunosuppression might be considered as an initial therapeutic strategy (III).

Use of viral load to monitor response to PTLD therapy and predict relapse: Although data are limited, in the short term, PTLD patients with high viral load as well as those receiving preemptive therapy, often demonstrate a fall and clearance of viral load coincident with clinical and histologic regression in response to interventions that include reduction of immunosuppression and adoptive immunotherapy (93,99). In contrast, some clinicians have observed that when rituximab is used, viral load measured in cellular blood components fell dramatically and remained low even in the face of progressive disease and disease relapse (100,101).

In pediatric patients, particularly those experiencing primary infection after transplant, asymptomatic intermittent or persistent viral load rebound occurs frequently with no

short-term consequences. Adult PTLD patients have been observed to relapse in the presence of persistently low viral load (101). However, recent data suggest that the sample type may influence the usefulness of viral load testing to monitor treatment response and predict relapse as plasma monitoring appears to correlate better with treatment response and relapse than monitoring in the cellular compartment (54,102). Further studies to confirm this observation are required (54,102).

A significant number of transplant recipients who experience primary EBV infection or EBV-positive PTLD have sustained elevation of EBV viral load after asymptomatic infection or resolution of EBV disease or PTLD (chronic high load carriers). The pathogenesis of this state is unknown. The detectable viral load appears to be predominantly in memory B cells with type 0 gene expression (103–105). Recent studies in thoracic pediatric chronic high load carriers suggest that these patients have high frequencies of activated but functionally exhausted EBV-specific cytotoxic T cells exhibiting unexpected immunopolarization. Whether this exhausted immune phenotype is also present in nonthoracic transplant recipients with chronic elevations in viral load and how this immune phenotype relates to PTLD risk is uncertain. Although a study in pediatric thoracic transplants suggest that patients who are chronic high viral load carriers (105) may be at significantly increased risk of late onset EBV-positive PTLD (106), this risk appears in part to be organ-specific with intermediate risks observed in intestinal transplants (107) and low risk in pediatric liver transplant patients from the same center (108). However, even among specific allograft types such as pediatric liver transplant recipients, reported long-term risks differ among centers (109,110). Additional data from prospective studies are required to determine allograft-specific long-term risks, the pathogenesis and evolution of this phenotype in relationship to PTLD risk in order to guide patient management and the usefulness of ongoing viral load monitoring in this setting.

Prognostic Indicators of PTLD

Several variables have been identified as indicators of prognosis in the management of PTLD. The extent to which findings can be generalized across centers is limited by the absence of a standardized approach to the pathologic diagnosis and treatment of PTLD. Table 4 summarizes some factors that have been associated with poorer outcomes.

Table 4: Factors associated with poorer outcomes from PTLD

Poor performance status
Multisite disease
Central nervous system disease
T or NK cell PTLD
Spindle cell PTLD
EBV-negative PTLD
The abnormal cells leading to PTLD of recipient origin as opposed to donor-origin
Coinfection with hepatitis B or C
Monoclonal disease
Presence of mutation of proto-oncogenes or tumor suppressor genes

Prognostic factors not always consistent among studies.

Summary of Key Recommendations/Statements

(1) Primary EBV infection and high or repetitive doses of antilymphocyte globulin represent the best-documented risk factors for the development of early PTLD (II-2).

(2) EBV serostatus should be determined on all transplant recipients and donors in order to identify the patients at high risk for PTLD development. Seropositive candidates <18 months of age should be considered seronegative for purposes of risk stratification (II-2). Patients seronegative prior to transplantation should be rescreened while on the waitlist and yearly after transplant to determine ongoing susceptibility to primary infection (III).

(3) The establishment of an international standard for EBV viral load assessment should reduce interlaboratory variability in reported results; this requires validation. In the interim, formal cross-referencing is required for interinstitutional result comparison (II-2). Serial monitoring of high risk (usually seronegative recipients) with EBV viral load as part of preemptive strategies for PTLD prevention is the best validated use of these assays (II-2); monitoring of low risk seropositive populations is not routinely recommended (II-3). The clinical benefit of EBV viral load assays for monitoring response to therapy, predicting relapse and for disease diagnosis is uncertain. Results obtained in these settings should be interpreted with caution; interpretation may be sample type dependent (II-3).

(4) Histopathology remains the gold standard for the diagnosis of PTLD (III).

(5) Antivirals ± immune globulin are sometimes employed as EBV prophylaxis after transplantation among EBV D+R– patients. There is insufficient evidence to support or refute this strategy (I). Where employed, a prophylaxis strategy similar to that for CMV may be considered (III).

(6) The use of preemptive strategies in high-risk populations may lower PTLD incidence rates; reduction in immunosuppression is the best documented intervention strategy (II-2). There are insufficient data to determine the efficacy of other intervention strategies such as antivirals, anti-CD20 antibody or adoptive immunotherapy (III).

(7) Additional data from prospective studies are needed to determine the significance of chronic, sustained elevations of EBV loads after transplantation (III).

(8) In patients who do not have rapidly progressive disease and who lack predictors of poor response to immunosuppression reduction, reduction of immunosuppression to the lowest tolerated level is recommended as initial therapy for early and late B cell PTLD (II-2). Other modalities of therapy depend in part of on the histopathologic characteristics of PTLD and location of lesions.
(9) In adult patients with PTLD, rituximab therapy should be considered as the next step in the treatment of CD20+ B cell PTLD after reduction in immunosuppression in patients who lack risk factors that predict rituximab failure (II-1).
(10) The use of chemotherapy should be considered for PTLD treatment after failure of reduction in immunosuppression in patients who have risk factors predicting poor response to rituximab monotherapy, patients who fail to achieve complete remission after initial rituximab therapy (II-1), and in the setting of T cell, Burkitt or Hodgkin lymphoma (III). Treatment of CNS disease requires special consideration (III).

Future Research Priorities

It is clear that several areas relating to EBV infection in the setting of transplantation are in need of further research. Additional research or consensus is needed to address and to enhance the levels of evidence for or against different aspects of the diagnosis, prevention and treatment of PTLD. A list of potential research targets include, but are not limited to the following:

(1) Understanding the pathogenesis of the full spectrum of PTLD.
(2) Standardization of the format used to report PTLD incidence trends.
(3) EBV vaccine evaluation for transplant candidates.
(4) Evaluation and standardization of EBV viral load measurement.
(5) Optimal use of antiviral ± immune globulin in patients at risk of EBV diseases posttransplantation.
(6) Enhancement of screening/diagnostic strategies to enhance the early detection of PTLD, beyond the use of viral load testing.
(7) Controlled trials of preemptive management modalities, including role of reduced immunosuppression with/without rituximab.
(8) Prospective studies of the significance of chronic viral load carriage.
(9) Continued research on optimal treatment for specific categories of PTLD, include the specific chemotherapy regimens with/without rituximab.
(10) Factors influencing susceptibility to EBV and EBV-related outcomes, including host and viral genetic variation.

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