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Posttransplant Lymphoproliferative Disorders Following Solid Organ Transplantation

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1. Introduction

Posttrasplant lymphoproliferative disorders (PTLD) represent a wide spectrum of pathologic and clinical manifestations that occur in the setting of depressed T-cell function and altered immune surveillance, such as is observed in the setting of solid organ transplantation (SOT). PTLD is a major contributor to long-term morbidity and mortality in this population and is the most common cancer observed in children following SOT. (Webber et al., 2006) Many cases are associated with Epstein-Barr virus (EBV) infection and most, but not all, are of host origin. (Petit et al., 2002, Taylor et al., 2005) This article reviews the pathology, epidemiology, risk factors, and clinical aspects of PTLD, and identifies the need for ongoing systematic study of complex biologic and therapeutic questions.

2. Pathology

PTLD encompasses a remarkable diversity of pathologic conditions. The most commonly used pathologic classification scheme is the World Health Organization (WHO) categorization, outlined in Table 1. (Swerdlow et al., 2008) Pathologic evaluation requires assessment of tissue architecture and cytologic features including immunophenotype; excisional biopsies are preferred and tissue samples should be submitted fresh rather than in formalin. It must be established if the PTLD is EBV-associated disease, for example by EBV-encoded RNA in situ hybridization (ISH) testing of diagnostic tissues. Cytogenetic studies assist with determination of clonality (immunoglobulin heavy [IgH] gene rearrangement; T-cell receptor gene rearrangement) and identify disease-associated chromosomal abnormalities. It is important to exclude other diagnoses including infection, inflammatory processes or rejection. Characteristics that suggest but are not pathognomonic of PTLD include enlarged nodules, mass lesions, lymphoid atypia, a very B cell rich infiltrate, extensive necrosis in the infiltrate and numerous EBV+ cells. (Dharnidharka, 2010) PTLD may be observed simultaneously at different sites in a patient, and a patient may have two different types of PTLD simultaneously or subsequently. (Blaes & Morrison, 2010)

3. Epidemiology and risk factors

The overall incidence of lymphoproliferative disease varies from 1 to 20% depending on the type of transplant and other risk factors. (Vegso et al., 2010) The frequency of PTLD is higher

in childhood; regardless of the transplanted organ the incidence is 2 to 3-fold compared to adults. The principal risk factors in the development of PTLD are the degree of immunosuppression and the EBV serostatus of the recipient. (Dharnidharka et al., 2001; McDonald et al., 2008; Opelz & Dohler, 2004) Similarly, younger age at transplant is a strong risk factor for PTLD, but likely reflects the proportion of recipients who are EBV-seronegative. Other risks described include past history of malignancy, the degree of HLA-compatibility and the occurrence and severity of acute rejection, the type of the transplanted organ, and the immunosuppressive drugs used. (Bakker et al., 2005b; Taylor et al., 2005; Tsao & Hsi, 2007)

3.1 Pathogenic mechanism of EBV

EBV is a polyclonal stimulator of B-cell proliferation. More than 90% of the world's population is infected with EBV and infected individuals remain lifelong carriers of the virus. (Rickinson & Kieff, 1996) The life cycle of EBV is outlined in Fig. 1. Primary infection occurs through the oropharynx, where the virus infects resting B cells. The expression of viral proteins induces polyclonal proliferation of infected B-cells and some of these differentiate into memory cells which carry the virus in a latent form. (Thorley-Lawson, 2001) Among immunocompetent hosts, control of virus spread and of unrestrained infected B-cell proliferation is maintained by the development of a specific immune response.

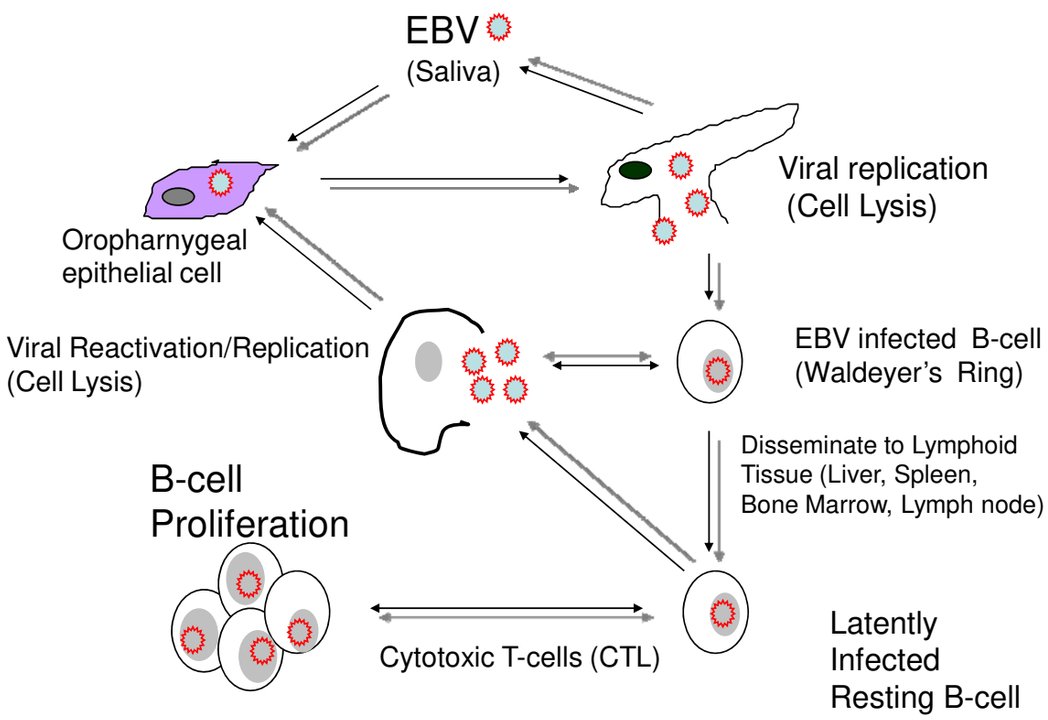


Fig. 1. EBV Life Cycle. (Courtesy of Dr. Thomas G. Gross)

Pathologic Categories of PTLD	
Category	**Description**
Early lesions • *Reactive Plasmatic Hyperplasia* • *Infectious mononucleosis-like*	• Lymphoid proliferations with preservation of normal tissue architecture. • In some cases just prominent follicular hyperplasia is seen. • Most of these early PTLD cases occur within a relatively short time after transplantation. • Polyclonal B cells. • More frequently in previously EBV-naive SOT recipients. • EBV often positive. (Swerdlow et al., 2008).
Polymorphic	• Morphology shows destruction of the underlying architecture of the tissue with perineural and blood vessel invasion. • Heterogeneous population with a full range of B-cell maturation and includes areas that seem more monomorphic, suggesting a continuum between polymorphic and monomorphic disease. • EBV often positive. • Monoclonal B cells, nonclonal T cells. • BCL6 somatic hypermutations may be seen. (Swerdlow et al., 2008).
Monomorphic ***B-cell neoplasms*** • *Diffuse large B-cell lymphoma* • *Burkitt lymphoma* • *Plasma cell myeloma* • *Plasmacytoma-like lesion* • *Other* ***T-cell Neoplasms*** • *Peripheral T-cell lymphoma, not otherwise specified* • *Hepato-splenic* • *Other*	• Classified according to the B-cell or T/natural killer (NK)-cell neoplasms described in the immunocompetent host. • Architectural effacement usually seen. • EBV +/-. • Clonal B cells or T cells. • Markers of oncogenes and tumor suppressor genes (eg, C-myc, N-ras, and p53) may be used to facilitate diagnosis in more complex cases. • Recurrent chromosomal abnormalities have been reported in some series of B-cell PTLD and may portend a worse prognosis. (Chadburn et al., 1997; Djorkic et al., 2006; Maecker et al., 2007; Swerdlow et al., 2008).
Classical Hodgkin lymphoma	• Primarily among renal transplant recipients. • Should fulfill the criteria for classical Hodgkin lymphoma recognized in the immunocompetent host as, Reed-Sternberg-like cells may be seen in early, polymorphic, and some monomorphic PTLD. • The expression pattern of EBV proteins (EBV-latency pattern) may aid in the diagnosis of cHL. • Architectural effacement usually seen. • EBV almost always positive. • IgH not easily demonstrated. (Pitman et al., 2006; Ranganathan et al., 2004; Rohr et al., 2008; Swerdlow et al., 2008).

Table 1. WHO classification of PTLD.

In a persistent infection, EBV antigen-specific T cells are maintained at a frequency of 1% to 5% of peripheral blood T cells to immune survey and eliminate reactivating/ proliferating infected B cells when they express the growth program. (Steven et al., 1996) T cell–mediated responses to the immunogenic proteins prevent outgrowth of EBV-infected B cells. In contrast, when T cell–mediated responses are impaired, uncontrolled proliferation of EBV-infected B cells will lead to the development of EBV-associated lymphoproliferative diseases. The disease is polyclonal in the beginning, however infected cells may then acquire genetic alterations that promote monoclonal lymphoma formation. The existence of EBV-unrelated PTLD supports the theory of a pathologic interaction between genetic aberration, viral oncogenes, impaired immunity and chronic antigen stimulation. (Nourse et al., 2011)

3.2 EBV+ vs EBV-

In children PTLD is usually induced by primary EBV infection which may occur via transmission from the allograft or the natural route via salivary secretion. Pediatric patients in particular are often EBV-seronegative, but around 90% will be positive at 6 months post-transplant. (Newell et al., 1996) However not all EBV-seronegative SOT recipients develop PTLD. (Guthery et al., 2003; Tsao & Hsi, 2007) In one pediatric report of pediatric liver transplant recipients, it was found that no seropositive patients developed PTLD following liver transplant; but 10.5% of patients who were seronegative prior to transplant developed PTLD. (Ho et al., 1988) The EBV status of the donor and the recipient is a relevant factor; the risk of PTLD increases 10 to 50-fold when the recipient is EBV-seronegative and the donor is EBV-seropositive. (McDonald et al., 2008, Tsao & Hsi, 2007) Of note, EBV cannot be detected in 15%–40% of cases of PTLD. PTLD was historically considered an early phenomenon with most cases reported within the first year following transplant and attributed to the more intense immunosuppression used for induction therapy or exposure of the EBV-seronegative host to the virus. (Faull et al., 2005; Webber et al., 2006).More recent data suggest that the median time is later, at approximately 3 years after SOT. (Evens et al., 2010; Ghobrial et al., 2010; Knight et al., 2009; Maecker et al., 2007) This is partly due to an increase in the diagnosis of EBV-unrelated disease. Multiple reports have shown that EBV-unrelated PTLD occurs later after SOT, most commonly after 3 to 5 years. (Dotti et al., 2002; Mamzer-Bruneel et al., 2000) The incidence of EBV-unrelated PTLD has been increasing and may be explained by changing immunosuppressive regimens, longer survival after SOT, and an improvement in the diagnosis. (Nelson et al., 2000) EBV-related and EBV-unrelated PTLD usually have different clinical manifestations. EBV-unrelated PTLD is usually a monomorphic disease with late manifestation and has historically carried a poor prognosis. (Dotti et al., 2002; Leblond et al., 1998; Nelson et al., 2000; Taylor et al., 2005) Recent data shows an improved response to treatment, which could be explained both by improvements in treatment strategies of the tumors and by improved supportive care. (Elstrom et al., 2006; Evens et al., 2010; Knight et al., 2009; Maecker et al., 2007)

3.3 Immunosuppression

In the absence of a reliable and reproducible method to measure intensity of immunosuppression, it is difficult to assess the effect of individual immunosuppressive agents on the risk of developing PTLD. In addition there is often a learning curve with new agents, such that the incidence of PTLD may be higher when an agent is first introduced to clinical care. (Nourse et al., 2011; Webster et al., 2005) It does appear that increasing T cell–specificity of immunosuppression is associated with a higher incidence of PTLD. The use of antibody preparations that deplete T cells has been identified as a risk factor for PTLD in early studies by both univariate and multivariate analyses. (Faull et al., 2005; Kirk et al., 2007; Opelz & Dohler, 2004; Yang et al., 2008) However, data from multiple transplant registries have demonstrated mixed results regarding a direct association between the use of Thymoglobulin and the risk of developing PTLD. (Marks et al., 2011) Quinlan et al. in a retrospective cohort study among 156,740 kidney transplant recipients reported that the use of antibody induction or antirejection therapies was not associated with PTLD risk, even when restricted to T-cell-based therapies, in contrast to earlier reports where there was an association between antibody induction therapy and risk of early onset PTLD. (Caillard et al., 2005; van Leeuwen et al., 2009) This difference may be related to different eras of

treatment and increasing experience with antibody-based induction therapies, leading to an attenuation of associated PTLD risks. (Quinlan et al., 2011)

In contrast, PTLD occurred 2 to 3-fold more frequently after receipt of OKT3 than with other drugs. (Opelz & Dohler, 2004; Taylor et al., 2005) Gajarski et al. report that as induction agents thymoglobulin and IL-2R antagonists had the lowest associated PTLD rates compared to OKT3, attributing this to its long-lasting depletional effects on CD-3 positive T-lymphocytes. (Gajarski et al., 2011) IL-2 receptor inhibitor antibodies (daclizumab, basiliximab) which specifically target activated T cells and are non-depleting, do not increase PTLD risk. Induction with alemtuzumab (anti-CD52 antibody), which depletes both T and B lymphocytes, was also not found to be associated with PTLD. (Caillard et al., 2005; Kirk et al., 2007; Opelz &Dohler, 2004) Shapiro et al. reported a small series using alemtuzumab as pre-conditioning with tacrolimus monotherapy in pediatric renal transplantation, with no cases of PTLD after a median time of follow up of 25 months. (Shapiro et al., 2007)

There are some controversies regarding the risk with the use of calcineurin inhibitors, which at this point in time continue as the pillars of maintenance regimens. Although early studies suggested the use of tacrolimus increases the risk of PTLD two to fivefold compared to cyclosporine, more recent studies suggest that if serum levels are monitored closely, there is no difference in risk of PTLD. (Dharnidharka et al., 2001; Guthery et al., 2003; Opelz & Dohler, 2004; Russo et al., 2004; Webster et al., 2005) Several groups have shown an increased risk of PTLD in kidney transplant recipients receiving tacrolimus compared with cyclosporine if they had not received antibody induction, but no difference in the recipients that had received induction. (Stojanova et al., 2011) In a recent report patients treated with tacrolimus after heart transplantation were at increased risk for the development of PTLD compared with patients treated with cyclosporine. (Dayton et al., 2011) Cyclosporine increases the risk mainly in higher doses (>6.6 mg/kg per day). (Boubenider et al., 1997) Both cyclosporine and tacrolimus inhibited apoptosis in a lymphoblastoid cell line, but it has been suggested that a difference in the risk could be due to a higher level of immunosuppression with tacrolimus compared to cyclosporine. (Stojanova et al., 2011)

Some studies have reported that sirolimus may decrease the incidence of PTLD. (Kauffman et al., 2005; Yakupoglu et al., 2006) Conflicting data from the UNOS registry study suggest that sirolimus is strongly associated with PTLD in kidney transplant recipients. The highest risk was limited to EBV-seronegative recipients with the clinical implication that sirolimus should probably be avoided in this population, especially in those who are already at higher risk for PTLD. (Kirk et al., 2007) Nee et al. also noted the same higher risk of PTLD with sirolimus, in a retrospective cohort of 53,719 kidney transplant recipients. (Nee et al., 2011)

It seems that antimetabolites such as azathioprine and mycophenolate mofetil used primarily as adjunct therapy alongside calcineurin inhibitors for preventing allograft rejection, are associated with a lower or not increased risk of PTLD. (Caillard et al., 2005; Kauffman et al., 2005) Mycophenolate mofetil may reduce PTLD risk by allowing the use of lower-dose calcineurin inhibitors. (Gajarski et al., 2011) Belatacept, is a new immunosuppressive agent that selectively blocks T-cell co-stimulation. In a recent study belatacept was associated with a greater risk of PTLD in the central nervous system (CNS) when compared with cyclosporine, especially in EBV-seronegative recipients and when used at more intensive doses. (Grinyo et al., 2010)

In addition to the type of the immunosuppressive drugs, the dosage, the combinations and the length of aggressive treatment influence the risk of PTLD, so the total immunosuppression exposure rather than induction alone may be a more accurate determinant of PTLD risk, and may be modified by choice of induction agent. (Gajarski et al., 2011)

3.4 Type of transplant

Most analyses suggest that the risk of PTLD is associated with the type of organ transplanted. The risk is lowest (1% to 3%) following renal and liver transplantation, moderate (1% to 6%) after heart transplantation, and highest after lung (4% to 10%), small intestine and multivisceral transplantation (>10% and may be as high as 20% for intestinal transplants). (Swerdlow et al; 2008) The reasons for these observed differences in incidence of PTLD include recipient factors, such as age and EBV serostatus, but also allograft factors, such as differing risk of transmitting EBV-infected B cells (in associated lymphoid tissue) with intestine and lung transplants compared with liver, heart, and kidney transplants, and the amount of immunosuppression required to prevent rejection. (Opelz & Dohler, 2004; Petit et al., 2002; Tsao & Hsi, 2007; Taylor et al., 2005) Among the differences regarding the type of organ transplanted is the time of presentation. Early-onset presentation of PTLD is characteristic for heart and lung transplantation, with nearly half of the cases appearing in the first year. In contrast among kidney transplant recipients, late-onset presentation is more common. This difference is due to higher doses of immunosuppression and induction treatment required following heart and lung transplantation. (Quinlan et al., 2011; Taylor et al., 2005) Another difference noted is the origin of the PTLD in renal transplantation-- PTLD of donor origin has been described. (Olagne et al., 2011)

3.5 Other risks

There are several other risks related to PTLD. A recent study reported that African American kidney transplant recipients are at lower risk for PTLD, irrespective of the recipient EBV serostatus. (Nee et al., 2011) Quinlan et al. found that non-Hispanic whites were at significantly higher risk of early-onset and late-onset PTLD than other racial or ethnic groups. (Quinlan et al., 2011) In the same study it was also demonstrated that CMV-seronegativity was associated with increased early-onset PTLD risk. (Quinlan et al., 2011) However, it seems that the role of CMV and HCV as risk factors for PTLD is controversial, as is that of herpes simplex or simian virus infections. (Nee et al., 2011; Tsao & Hsi, 2007) An increase in the risk of PTLD has been described in the presence of specific cytokine gene polymorphisms. (Taylor et al., 2005; Tsao & Hsi, 2007) Malignancy and autoimmunity are also associated with PTLD. Patients with PTLD are more likely to have a history of pre-transplant malignancy than those without PTLD. (Caillard et al., 2005; Nee et al., 2011) Transplantation due to autoimmune hepatitis and primary biliary cirrhosis carries a higher risk of PTLD likely related to chronic immunologic stimulation. (Shpilberg et al., 1999). Transplantation due to cystic fibrosis and Langerhans-cell histiocytosis carries a higher risk of PTLD due to a higher incidence of refractory rejection. (Cohen et al., 2000; Newell et al., 1997) In addition, Zimmermann et al. found that liver recipients who received steroids before transplant for immunological disorders are at particularly high risk to develop PTLD. (Zimmermann et al., 2010)

The role of HLA mismatch in the risk of PTLD is controversial. Among kidney transplant recipients, HLA-B mismatches have been reported to increase the risk of lymphoma in the allograft, whereas HLA-DR locus mismatches may increase the risk of non-Hodgkin lymphoma in the allograft and the central nervous system. (Bakker et al 2005b; Opelz & Dohler 2010) Reshef et al. suggest that the HLA-A26, B38 haplotype is mainly responsible for predisposition to PTLD, at least among Caucasian recipients, as an independent risk factor. (Reshef et al., 2011) Other studies have been unable to confirm a role for HLA mismatches and an increased risk of PTLD. (Nee et al., 2011; Quinlan et al., 2011)

The assessment of risk factors for PTLD is complicated by the complex nature of the disease and the variability among studies with respect to disease definitions, immunosuppression protocols and the length of follow up. Methods of screening for and monitoring of EBV have improved over time, as well as the supportive therapy for transplant procedures and their complications. As a consequence, there is a tendency toward lower incidence of PTLD; it was observed to be less likely to develop in patients who received their allograft after 1996, than those who received allograft before 1996. (Dayton et al., 2011; Marks et al., 2011; Tsao & Hsi 2007)

4. Diagnosis

4.1 Clinical presentation

A high index of suspicion is required for a timely diagnosis of PTLD. Patients often present with relatively benign findings before developing more significant symptomatology. Rarely, SOT recipients may present with so-called fulminant PTLD. Other EBV-associated diseases must be differentiated from PTLD, although the initial management is similar. (Gross, 2009) There are two forms of presentation: early-onset and late-onset PTLD. Early-onset PTLD occurring within 2 to 3 years of transplantation is more common among pediatric SOT recipients because of their risk for primary EBV infection. (Opelz & Dohler, 2004) Early-onset disease is more likely to involve the allograft and present with declining allograft function, excepting heart allografts, in which direct involvement by PTLD is rare. (Bakker et al., 2005a) The major differential diagnostic considerations include allograft rejection and infection. Later-onset disease is more likely to be EBV negative and to include T/NK-cell disease, to involve extranodal sites (especially GI sites) or to present with dissemination. (Guthery et al., 2003; Ho et al., 1988; Leblond et al., 1998; Nelson et al., 2000; Steven et al., 1996) T/NK-cell disease is rare although it may be increasing, is clinically aggressive and is more often EBV negative (two-thirds of cases). (Azhir et al., 2009; Gupta et al., 2010; Miloh et al., 2008; Williams et al., 2008; Yang, et al 2008)

Virtually no site is exempt from PTLD involvement. Outside the allograft, common areas affected include lymphoid tissues, GI tract, lung, kidney and liver. Patients may present with constitutional symptoms (fever, poor weight gain, rash), mono-type illness, lymphadenopathy, and organ dysfunction. A frequent presentation in early-onset disease is adenotonsillar involvement with associated sore throat and obstructive symptoms (new onset snoring or mouth breathing). (Allen et al., 2005) Involvement of the GI tract may present with feeding intolerance, vomiting, diarrhea, protein losses, bleeding, intussusception, or obstruction. Perforation may occur at presentation or immediately following initiation of therapy in the presence of transmural lesions. New-onset anemia or hypoalbuminemia may indicate GI involvement. (Selvaggi et al., 2006) Lung disease may

result in unexplained cough, wheezing and respiratory insufficiency or asymptomatic nodules. Liver disease may present as an unexplained increase of serum transaminases, diffuse hepatitis or nodular lesions. Other signs are joint pain and auto-immune cytopenias. PTLD of the central nervous system, isolated or as part of multiorgan disease, may be as high as 30%, compared to only 1% among non-Hodgkin lymphomas of the non-transplanted population. (Maecker et al., 2007; Taylor et al., 2005) Patients may present with headache, seizures, or focal neurologic findings.

Some general comments may be made in regard to clinicopathologic correlation. The early-onset PTLD lesions are generally EBV-related and typically fall into the category of early lesions or polymorphic PTLD. In children and adults with late-onset PTLD, EBV-related early lesions or polymorphic PTLD may still be observed, but there is a greater percentage of monomorphic and EBV-unrelated diseases, especially in adults. (Allen, 2010) However, Quinlan et al. in a retrospective cohort study of kidney transplant recipients, reported that early-onset PTLD was more likely to be monomorphic than polymorphic (48.2% vs. 41.6%, with 10.2% of unknown), and late-onset PTLD was even more likely to be of monomorphic pathology (55.9% vs. 31.4%, 12.7% unknown). Early onset PTLD was predominantly of B-cell origin, late-onset PTLD showed a slightly higher proportion of T-cell PTLD (64.3% B-cell vs. 9.7% T-cell, 25.9% unknown). (Quinlan et al., 2011)

4.2 Clinical evaluation

Initial assessment includes a full physical examination with meticulous assessment for lymphadenopathy, and a risk adapted approach to the selection of screening evaluations and EBV-specific monitoring.

4.2.1 Screening tests

Screening tests include a complete blood count with differential, chemistry panel to assess for tumor lysis syndrome, allograft function screening, CMV by PCR and other viruses (human immunodeficiency virus type 1 & 2, hepatitis B, hepatitis C). Imaging evaluation is essential and is directed initially by the location of suspected lesions and prior radiographic studies of each patient. Ultrasound is effective for initial imaging in patients with suspected abdominal/pelvic or soft-tissue PTLD. CT is used in evaluation for neck and chest disease and in staging with a suspected or confirmed diagnosis. Head CT or MRI should be included to assess relevant symptoms and in staging evaluations. In patients with abdominal symptoms, upper and lower endoscopy should be considered early as lesions may be missed on routine imaging. (Allen, 2010) The role of [18F]2-fluoro-2-deoxyglucose (FDG)-positron emission tomography (PET) in the diagnosis of equivocal lesions, staging, and response assessment of PTLD is currently being defined. (von Falck, et al 2007) Bakker and colleagues demonstrated additional extranodal sites on PET not appreciated on computed tomography (CT) in 50% of patients and concordance of PET response with outcome. (Bakker et al., 2006) However, false positives have been described when PET is used for disease monitoring in children and for the evaluation of lung lesions. (McCormack et al., 2006; Rhodes et al., 2006) Recommended diagnostic procedures are similar to other non-Hodgkin lymphoma patients, and staging is completed using the Ann Arbor staging system. Diagnosis must be confirmed by biopsy. See Fig. 2



Fig. 2. Diagnostic and staging procedures for PTLD.

4.2.2 EBV-specific tests

Serology is critical to categorize the serostatus of the donor and recipient at the time of transplant. Serology following SOT is unreliable as a diagnostic test in immunocompromised patients as they have marked delay in their humoral response. Primary EBV infection, rather than EBV reactivation, is responsible for the majority of PTLD cases in children, and early diagnosis may be prompted by serial measurements of EBV DNA in peripheral blood after transplant. An increasing EBV DNA load may be an early sign of PTLD, requiring further evaluation and closer monitoring. (Allen, 2010)

EBV-DNA may be measured in the plasma, peripheral blood mononuclear cells (PBMC) and in the whole blood by quantitative PCR assays. Testing done with plasma measures the cell-free fraction only and may lead to an underestimation; however the detection of plasma EBV-DNA is a highly specific test with a high positive predictive value (100%). One possible explanation is that free viral DNA is released from latently infected cells, but most notably tumor cells undergoing apoptosis. Tsai et al. found EBV-DNA measured in plasma had comparable sensitivity and improved specificity compared to whole blood for diagnosis of EBV-related PTLD. (Tsai et al., 2008) Measuring intracellular EBV (PBMC) is considered to be less specific, due to the high rate of false positivity. In patients with infectious mononucleosis viral DNA is detectable in serum for only 7 days after symptom onset and then remains detectable in the cellular fraction. Whole blood assays detect EBV-DNA present in the cells and in the plasma. Whole blood assays may be better used when screening for PTLD, and plasma assays may be better for the evaluation after therapy of PTLD. The time intervals for routine monitoring and duration of monitoring may vary depending on the type of transplant and individual risk factors. Clinicians must be aware that PTLD may be EBV-unrelated, and that EBV-related disease may develop or recur in the absence of an increasing EBV-DNA load. (EBV Work Group, Cincinnati Children´s Hopsital Medical Center, 2011; Tsai et al., 2008) Adjunctive tests to assess the resiliency of the immune system have been investigated, including T-cell restoration or EBV-specific T-cell response. Low T-cell response and high EBV-DNA load indicates high degree of immunosuppression and an increased PTLD risk, which have to lead a modification of immunosuppression and further examination of the

patient. Testing of EBV in the CSF is often used to assist in the diagnosis of CNS PTLD and avoid the need for more invasive biopsy procedures. (Preiksaitis, 2009)

5. Treatment

Treatment strategies for PTLD must be tailored to individual clinical contexts and require input from an interdisciplinary team including a transplant specialist, an oncologist and an infectious disease specialist. Decisions are based on multiple factors including disease presentation, EBV status, patient comorbidities and performance, risk of rejection, type of organ graft, and the drugs used for immunosuppressive therapy. Where impairment of the transplanted organ can lead to the death of the patient, the protection of the organ graft is a critical factor in planning therapy.

5.1 Primary prevention

5.1.1 Donor EBV serologic status

Avoiding EBV-positive donors in EBV-naive recipients to limit primary infection may prevent PTLD. However, only a very small proportion of donors are EBV negative and this approach cannot be applied universally. (Lazda, 2006)

5.1.2 Antiviral therapy

Increased attention is focused on the prevention of EBV-related PTLD through the use of antiviral therapy. Antiviral agents, such as acyclovir and ganciclovir, are nucleoside analogues that act by inhibiting the replication of EBV DNA through inhibition of viral DNA polymerase. Their activity is dependent on intracellular phosphorylation by virally encoded thymidine kinase. Ganciclovir seems to be more potent and to have a prolonged effect. Antiviral therapy may help prevent PTLD in EBV seronegative SOT recipients. Cells that are latently infected with EBV and cells of EBV-driven lymphomas do not express thymidine kinase. Therefore, these agents would not be expected to be effective in EBV-positive PTLD; however, they do limit the lytic replication of EBV-infected cells, reduce the viral load, and prevent infection of memory B cells and germinal center cells. (Taylor et al., 2005)

Limited evidence is available to address the efficacy of this therapy in the prevention of PTLD and most studies are retrospective, with inherent limitations and contradicting results. Prophylactic intravenous ganciclovir after liver transplant in children has been associated with decreased incidence of PTLD, perhaps due to reduction in the number of latently infected B lymphocytes. (Thorley-Lawson & Gross 2004) Marks et al. report that the overall incidence of PTLD was significantly lower when antiviral prophylaxis was used than when it was not used or not reported. They suggest that antiviral drug therapy may affect PTLD risk, even when the primary purpose is prevention of CMV infection, as prophylaxis may also inhibit EBV reactivation and primary infection, or progression to B-cell transformation and PTLD. (Marks et al., 2011) A retrospective study of 3,393 heart transplant recipients demonstrated that PTLD incidence did not increase after OKT3 or ATG induction therapy when acyclovir or ganciclovir were administered prophylactically. (Crespo-Leiro et al., 2007) However, Opelz et al. found in a retrospective study of 44,828 kidney transplant recipients that ganciclovir or acyclovir had no impact on the development of PTLD. (Opelz et al., 2007)

There have been small studies that have attempted to use arginine butyrate, an amino acid derivative, which induces EBV thymidine kinase expression in latently infected B cells, in conjunction with antivirals in the therapy of PTLD. (Oertel & Riess 2002; Perrine et al., 2007) This therapy is currently only available in clinical trials.

5.1.3 Passive and active immunization

An absence of antibody against at least one of the Epstein-Barr nuclear antigens (EBNA) in EBV-seropositive organ transplant recipients has been associated with the subsequent development of PTLD. There are also many patients that undergo primary EBV infection following transplantation and fail to develop anti-EBNA antibodies. Thus, the absence of anti-EBNA antibodies appears to correlate with an increased risk of developing PTLD. Increasing levels of anti-EBNA antibodies, including those introduced through transfusion, have correlated with decreasing EBV load. All of these data suggest a potential role for antibody in controlling EBV infected cells. (Green, 2010) The potential prophylactic benefit of CMV-IVIG against the development of EBV-related PTLD in pediatric liver transplant recipients has been evaluated in a randomized multicenter trial. Statistically significant differences were not observed, but rates of EBV disease and PTLD were somewhat lower in recipients of CMV-IVIG than in those who received placebo. (Green et al., 2006) One study found that anti-CMV immunoglobulin was effective in the prevention of early-onset PTLD in kidney transplant patients, but not in the prevention of late-onset PTLD. (Opelz et al., 2007)

EBV vaccination may be effective in PTLD prevention, especially in EBV-seronegative pediatric transplantation candidates, but the vaccines to date have had no reliable effects on the development of cell-mediated immunity. The role of this therapy is still controversial, and it is not commercially available. (Posfay-Barbe & Siegrist 2009)

5.2 Secondary prevention

5.2.1 Intervention after rising EBV-DNA load

Development of EBV-associated PTLD is usually preceded by increased levels of EBV-DNA load in peripheral blood, typically from 2 to 16 weeks before diagnosis. (Rowe et al., 2001; Schubert et al., 2008) EBV-DNA load is higher among SOT recipients subsequently diagnosed with PTLD than those who remain disease free.(Bingler, et al 2008; Webber et al., 1999) There is a trend towards higher EBV loads following primary infection compared with reactivation (Kenagy et al., 1995), consistent with the clinical observation of higher risk of PTLD following primary infection. (Allen et al., 2005; Ho et al., 1988) Between 60% and 80% of EBV-negative children seroconvert within 3 months of transplant (Green & Webber, 2007) and a small population maintain high viral loads after primary EBV infection or EBV-associated PTLD. (Bingler et al., 2008; Green & Webber, 2007) It is common practice to perform biweekly or monthly EBV-DNA load monitoring by quantitative PCR and/or immune function monitoring in the immediate post transplantation period with varying clinical practice for reduction of immunosuppression (RI), use of antiviral agents, use of monoclonal anti-CD20 (Rituximab) or cellular therapy in the event of rising EBV-DNA load. Which form of pre-emptive therapy is best to prevent PTLD is unclear at this time.

In pediatric liver transplant recipients, monitoring EBV-DNA loads in the first 6 months after transplant with RI and initiation of ganciclovir at the time of rise in EBV-DNA load

resulted in a 50% reduction in the incidence of PTLD. (McDiarmid et al., 1998; Stevens, et al., 2002) In 73 pediatric liver transplant patients treated during 2001– 2004, the incidence of PTLD was reduced from 16 to 2% by RI in individuals with a high EBV load. (Lee, et al 2005) Series of pediatric intestinal transplant recipients reported similar results. (Green et al., 2000; Krieger, et al 2000) Rituximab is generally well tolerated and rapidly induces the depletion of mature B-lymphocytes, reducing the compartment of EBV infected cells, with an associated normalization of the viral load. It has been successfully used as a preemptive treatment for PTLD, specifically in the HSCT population. (Comoli et al., 2007; Styczynski et al., 2009) A preliminary report of adult cardiac recipients given rituximab as pre-emptive therapy, showed that only one patient (n=251) developed PTLD. (Choquet et al., 2011) This approach merits further evaluation for widespread application.

5.2.2 Cellular therapy

Recognizing the critical role of EBV-specific cytotoxic T-cells (CTLs) in immune surveillance, cellular therapy is considered both a preventive and therapeutic strategy with initial experience in EBV-related PTLD following HSCT. (Rooney et al., 1998) The implementation of this therapy in SOT it is more problematic where PTLD is primarily of recipient origin and often occurs in patients who are EBV seronegative prior to transplant. Some of the problems with the use of individual-based EBV-specific CTLs are the cost and the processing time required to generate the cells. Moosmann et al. recently reported a system that allowed the rapid isolation and generation of clinical-grade EBV-specific CTLs for the treatment of HSCT-related PTLD. (Moosmann et al., 2010) An alternative is to use allogeneic CTLs closely matched with human leukocyte antigen (HLA) from donors who are EBV seropositive. In a phase II multicenter clinical trial, relapsed and refractory PTLD patients were treated by weekly intravenous allogeneic CTL infusions for 4 weeks. No adverse effects were observed and the response rate was 64% at 5 weeks and 52% at 6 months in 33 patients. (Haque et al., 2007) Commercial banks of EBV specific CTLs generated from the peripheral blood of EBV-positive blood donors are in development. In the SOT setting, there is often the need to continue immunosuppression and this impairs the efficacy of adoptive T-cell therapy. In a effort to solve this problem, two groups have generated EBV-based CTLs resistant to calcineurin inhibitors and have shown efficacy of this approach without requiring a reduction in immunosuppression. (Brewin et al., 2009; Haque et al., 2007) EBV specific CTL therapy is now recommended for persistent or progressive EBV driven polymorphic or monomorphic PTLD. (NCCN Guidelines Version 4.2011)

5.3 Therapy

5.3.1 Reduction in immunosuppression (RI)

The initial approach to managing patients with PTLD is RI, which might restore CTL function and elicit a favorable response in EBV-positive PTLD. The dosage of immunosuppressive drugs must be reduced by 25%–50% of the normal therapeutic whole blood level. This approach is most effective for early lesions and polymorphic disease and for those patients diagnosed early following transplantation. (Cheema et al., 2008; Nelson et al., 2000; Taylor et al., 2005) The long term remission rate of early lesions and polymorphic PTLD treated with RI is 40%–86% in children but considerably lower in adults. (Carbone, et al 2008, Green, et al 1999; Taylor et al., 2005) In many cases additional therapy is required

(Reshef R et al., 2009). Lack of response to RI has been associated with elevated LDH at presentation, organ dysfunction, late-onset PTLD, and multiorgan involvement. The median time to response of RI is 2 to 4 weeks; patients with aggressive disease or high tumor burden often warrant more immediate treatment. The risk of graft rejection must be considered before RI. (Caillard, et al 2005) The risk of acute rejection may be reduced by the administration of corticosteroids, which are also important components of most chemotherapy regimens for PTLD. (Taylor et al., 2005) RI is generally well tolerated among liver and renal transplant recipients with non-invasive screening available for detection, and decreased risk and relative tolerability, of organ rejection; indeed complete withdrawal of immunosuppression may be possible in selected liver transplant recipients. (Londono et al., 2010; Tsai et al., 2001) RI strategy is not standardized. Differences of aggressiveness and duration of RI, amount of time to evaluate response prior to initiating second-line treatment, and patient factors (type of transplant, extent of disease, time from transplant and immunosuppression history), may explain reported differences in outcomes.

5.3.2 Chemotherapy and rituximab

Chemotherapy and/or rituximab are commonly used when RI fails to control the disease, and as an initial therapy for aggressive, monoclonal PTLD. Low dose chemotherapy may decrease toxic complications seen in immunosuppressed patients, but may lead to higher relapse rates. (Gross, et al 2005) The use of anti-CD20 antibody therapy (rituximab) as a single agent has been associated with overall response rates of 37–69% and is potentially beneficial and less toxic than systemic chemotherapy. (Blaes et al., 2005; Orjuela, et al 2003) Rituximab/chemotherapy (R/C) combination has been examined in an attempt to improve response rates. (Evens et al., 2010) Gupta et al., reported an overall response rate of 100% in the patients treated with R/C combination (low dose of chemotherapy) with a recurrence rate of only 12% and 2-year failure free survival of 80%. (Gupta et al ., 2010) Orjuela et al., in a pilot multi-center study of pediatric patients treated with cyclophosphamide, prednisone and rituximab, report an overall response rate of 100% (five complete response [CR] and one partial response) with a median follow-up of 12.5 months. (Orjuela, et al 2003) A recent single-center study reported a two year OS and EFS of 85.7% and 57%, respectively, with a combination of rituximab and a milder chemotherapy regimen. (Gallego et al., 2010) Trappe et al have recommended a risk stratified approach based on upfront response to 4 weekly doses of rituximab: patients with a complete response (CR) to rituximab continued to receive an additional 4 doses of rituximab alone whereas patients with less than a CR subsequently received rituximab plus CHOP chemotherapy for four cycles with growth factor support. Using this approach, the overall response rate was 89%. (Trappe et al., 2009) Rituximab as a single agent is a consideration for many patients, with the use of combination chemotherapy for progressive or relapsed disease or for patients with concomitant allograft rejection.

Some groups have tried to identify at diagnosis patients likely to have a poor response to rituximab monotherapy. One study suggested that good response in late PTLD was only seen when rituximab was used after either surgical resection or radiotherapy (Dotti et al, 2001) and, in another, EBV-negative PTLD did not respond to rituximab and subsequently required chemotherapy (Oertel et al, 2005). The recommendation of the British Committee for Standards in Haematology (BCSH) and the British Transplantation Society (BTS) guidelines for adults is that rituximab plus chemotherapy should be used for patients who

fail to respond within 8 weeks of rituximab plus RI and it should be considered immediately at any stage following diagnosis for patients with clinically aggressive disease or those with critical organ compromise (Parker et al, 2010).

Chemotherapy is used as a first line therapy for Burkitt lymphoma, T-cell disease and for most cases of Hodgkin lymphoma. Burkitt lymphoma seems to respond well to immediate aggressive chemotherapy. (Picarsic et al., 2010) Hodgkin disease is often associated with EBV and responds well to standard therapy. (Bierman et al., 1996) Available literature suggests that T -cell PTLD is clinically aggressive and may be associated with a poor prognosis. (Azhir et al., 2009; Gupta et al., 2010, Miloh et al., 2008; Williams et al., 2008; Yang, et al 2008)

5.3.3 Isolated CNS PTLD

CNS disease has been associated with significantly inferior survival, compared with non-CNS PTLD. (Buell et al., 2005; Knight et al., 2009; Maecker et al., 2007) It is more commonly associated with EBV. (Choquet et al., 2008) The optimal therapy for CNS PTLD is not known and proposed treatment approaches include high-dose methotrexate-based therapy, intrathecal chemotherapy and intrathecal rituxumab. (Bonney et al., 2011; Choquet et al., 2008; Taj et al., 2008; van de Glind et al., 2008)

5.3.4 Surgery and radiation

Resection of a total solitary lesion may be curative, but is usually combined with RI. (Allen, et al 2001) Radiation is rarely used but may be considered when rapid local responses are required (eg. airway compression) and in some cases of CNS PTLD.

5.3.5 Relapsed/refractory disease

There is no standard recommendation for relapsed or refractory PTLD. Trappe et al. have shown that rituximab may still lead to response in patients who fail chemotherapy, and chemotherapy might lead to response in patients with failure after rituximab. (Trappe et al., 2007a, 2007b) In addition, autologous hematopoietic stem cell transplantation has been reported as a viable treatment option for relapsed PTLD. (Amar et al., 2006) The role for cellular therapy in this context was reviewed above.

6. Prognosis

Although there has been improvement over time for patient and graft survival, outcomes after PTLD remain suboptimal. Survival after PTLD differs by recipient age and allograft type. Outcomes for children with PTLD are much better than for adults. (Dharnidharka, 2010) Outcomes are highest for recipients of renal and liver allografts, with survival rates for children reported at 89% and 80% respectively (Fernandez et al., 2009; McDonald et al., 2008), but significantly lower for other types of transplant, 67% at five years in heart transplant recipients, 54% and 42% at 3 and 5 years respectively in lung transplant recipients. (Cohen et al., 2000; Webber et al., 2006) Further, the overall survival according to stage is 80% for stages I/II, 61% for stage III, 20% for CNS involvement and less than 20% for BM involvement. The overall survival success for mild to moderate PTLD cases seemed similar across the various treatment options. (Maecker et al., 2007)

7. Conclusions

Patients with PTLD present a multifaceted clinical challenge balancing cure, allograft function and other co-morbidities. The epidemiology of PTLD appears to be changing with the median time of presentation trending later and the percentage of monomorphic and EBV negative disease increasing concomitant with changing immunosuppression practices, improved identification of patients at risk, and early introduction of RI. Even with these advances some patients have a good initial response to therapy without a long remission, and the mortality rates remain high. There is an ongoing need for coordinated care programs and clinical practice guidelines for a consistent approach to care and to further research programs. Novel therapeutic approaches aimed at restoration of immune surveillance should continue to be examined.

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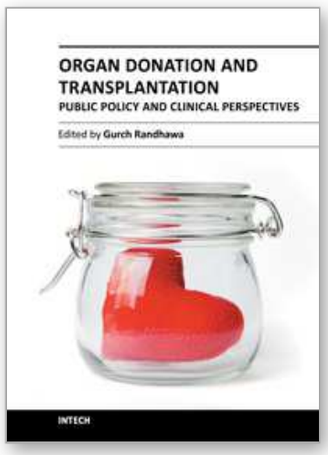
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Transplantation has succeeded in prolonging the lives of those fortunate enough to have received the gift of a body organ. Alongside this life-saving development, there lies another sadder side to the story - there are not enough organs to meet the ever increasing demand. This not only places an increasing emotional and physical burden among the waiting patients and families but heaps a great financial burden upon health services. This book provides an analysis and overview of public policy developments and clinical developments that will hopefully ensure an increased availability of organs and greater graft survival. Medical, policy, and academic experts from around the world have contributed chapters to the book.

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