bjh guideline

Diagnosis of post-transplant lymphoproliferative disorder in solid organ transplant recipients – BCSH and BTS Guidelines

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Summary

A joint working group established by the Haemato-oncology subgroup of the British Committee for Standards in Haematology (BCSH) and the British Transplantation Society (BTS) has reviewed the available literature and made recommendations for the diagnosis and management of post-transplant lymphoproliferative disorder (PTLD) in adult recipients of solid organ transplants. This review details the risk factors predisposing to development, initial features and diagnosis. It is important that the risk of developing PTLD is considered when using post transplant immunosuppression and that the appropriate investigations are carried out when there are suspicions of the diagnosis. These must include tissue for histology and computed tomography scan to assess the extent of disease. These recommendations have been made primarily for adult patients, there have been some comments made with regard to paediatric practice.

Keywords: Transplant, lymphoproliferative disease, diagnostic haematology, EBV.

Post-transplant lymphoproliferative disorder (PTLD) is a relatively common malignancy post-transplantation and an incidence as high as 10% in solid organ transplant recipients has been reported (Burns & Crawford, 2004). In adult transplant recipients, it is the second most common malignancy after skin cancer, and in children the most common post-transplant malignancy (Boubenider *et al*, 1997; Feng *et al*, 2003). The USA United Network for Organ Sharing (UNOS) Ad Hoc Disease Transmission Advisory Committee recently reported a 5-year cumulative incidence of PTLD for paediatric recipients of 3·26% as against adult recipients of 0·91% for the 5 years 2003–2007 (Ison & Nalesnik, 2009). The overall mortality reported in the literature, which may not be representative of current clinical experience, is approximately 50% (Leblond *et al*, 1995; Newell *et al*, 1996; Opelz & Döhler, 2004).

In case series from Europe and the USA, approximately 85% of PTLD was found to be of B-cell origin (Morrison *et al*, 1994; Leblond *et al*, 1995) and over 80% was associated with Epstein Barr Virus (EBV) infection (Allen, *et al* 2001). Approximately 15% of PTLD are of T-cell lineage, and of these, about 30% are associated with EBV (Hoshida *et al*, 2001). Rarely, other haemopoietic lineages, for example natural killer (NK) cells, will result in PTLD (Allen, *et al* 2001, Hoshida *et al*, 2001). It was recognised early in the history of transplantation that EBV was responsible for a high proportion of B cell PTLD (Crawford *et al*, 1980; Purtilo, 1980). In other parts of the world, for example the Far East, the proportion of T-lymphocytic PTLD is much higher than in the West and is as high as 40% due to the prevalence of the Human T cell leukaemia virus (HTLV) (Hoshida *et al*, 2001).

PTLD may occur at any time after transplantation. However, from the Collaborative Transplant Study database, the risk of developing PTLD appears to be greatest within the first year post-transplantation. The incidence was reported as 224/100 000 in the first year, 54/100 000 in the second year and 31/100 000 in the sixth year post-transplantation (Opelz & Döhler, 2004). PTLD represents a spectrum of disease that ranges from an indolent (polyclonal) lymphoproliferation usually seen early post-transplant, which is EBV positive, and often resolves on reduction of immunosuppression, to malignant aggressive lymphoma, which is rapidly fatal without combination chemotherapy. Treatment decisions are made based on histological subtype, grade, stage and site of the tumour as well as an assessment of the patient's clinical state, including transplanted (and other) organ function, and capacity to tolerate therapy.

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Table I. Classification of evidence levels.

I	a. Evidence obtained from meta-analysis of randomized controlled trials
	b. Evidence obtained from at least one randomized controlled trial
II	a. Evidence obtained from at least one well-designed controlled study without randomization
	b. Evidence obtained from at least one other type of well-designed quasi-experimental study*
III	Evidence obtained from well-designed non-experimental descriptive studies, such as comparative studies, correlation studies and case studies
IV	Evidence obtained from expert committee reports or opinions and/or clinical experiences of respected authorities

*Refers to a situation in which implementation of an intervention is out with the control of the investigators, but an opportunity exists to evaluate its effect.

Method

A joint writing group was convened by the British Committee for Standards in Haematology (BCSH) haemato-oncology task force and British Transplantation Society (BTS) and has compiled 'Guidelines on the Surveillance, Diagnosis and Management of Post-Transplant Lymphoproliferative Disorders in Adult Solid Organ Transplant Recipients' (Parker *et al*, 2009). The recommendations were made using the Agree instrument (http://www.agreecollaboration.org) and were further reviewed by members of the BCSH sounding board and British Transplant Society, representing practice in both teaching and district hospitals. The levels of evidence used were those of the US Agency for Health Care Policy and Research (see Tables I and II).

Biology of post-transplant lymphoproliferative disorders

Although PTLD are histologically and clinically heterogeneous, there is abundant evidence that Epstein-Barr virus (EBV) plays a critical role in the biology of the condition for many cases (Dolcetti, 2007).

EBV is a gamma herpes virus, which is a potent transforming agent, known to produce blastic transformation and uncontrolled proliferation in B-cells. In healthy individuals EBV infection is normally controlled by both antibody-based and CD4 and CD8 T-cell mediated immune responses. In non-immunosuppressed individuals the infection can be asymptomatic, or result in a benign, self-limiting proliferation of lymphoid cells, known as infectious mononucleosis (IM).

Table II. Classification of grades of recommendations.

A. Requires at least one randomized controlled trial as part of a body of literature of overall good quality and consistency addressing specific recommendation. (Evidence levels Ia, Ib)
B. Requires the availability of well conducted clinical studies but no randomized clinical trials on the topic of recommendation. (Evidence levels IIa, IIb, III)
C. Requires evidence obtained from expert committee reports or opinions and/or clinical experiences of respected authorities. Indicates an absence of directly applicable clinical studies of good quality. (Evidence level IV)

EBV genes are found in more than 90% of B-cell PTLD occurring in the first year after transplantation.

In EBV-positive PTLD (particularly the monomorphic cases) the virus is commonly monoclonal, indicating its incorporation at an early stage of clonal expansion. EBV seronegativity before transplant and primary EBV infection post-transplantation both increase the risk of developing PTLD. A decrease in EBV-specific cytotoxic T lymphocytes (CTL) and, in the majority of studies, an increase in EBV viral load, are both strongly associated with PTLD development (Sherritt *et al*, 2003; Kremers *et al*, 2006; Sebelin-Wulf *et al*, 2007). In seropositive solid organ transplants the majority of PTLD cases are derived from recipient cells, suggesting EBV reactivation, but some are related to a proliferation of donor cells (Capello *et al*, 2009).

Reduction of immunosuppression can lead to regression of PTLD due to restoration of cytotoxic T-cell function, and treatment with autologous EBV-specific CTL can result in reduced viral load and a degree of tumour control (Comoli *et al*, 2005). In addition, adoptive immunotherapy with CTLs derived from a bank of EBV seropositive blood donors shows promise in controlling PTLD in patients who have failed conventional therapy, with the advantages of ease of generation and deployment over conventional donor derived expansion procedures (Haque *et al*, 2002, 2007).

In approximately 20% of PTLD cases the cells are EBV negative (Nelson *et al*, 2000). The histogenesis of these lesions is unclear, but there are clinical differences between EBV-positive and EBV-negative PTLD. The latter occur late after transplantation and a higher percentage fall into the monomorphic category (Leblond *et al*, 1998; Dotti *et al*, 2002). Whilst these may represent 'co-incidental' lymphomas as seen in non-transplanted patients, some EBV-negative cases will respond to reduction of immunosuppression.

A subset of PTLD cases are derived from T-cells or NK cells (Kwong *et al*, 2000; Lau *et al*, 2004; Tsao *et al*, 2004; Tsai *et al*, 2005). The majority (60–90%) of T-cell PTLD are EBV negative (Dockrell *et al*, 1998), whilst most NK cell PTLD are EBV positive. The precise mechanisms by which these T-cell or NK cell proliferations come about have yet to be elucidated.

Histology of post-transplant lymphoproliferative disorders

Handling of specimens

Pathological specimens should be handled according to standard lymphoma protocols as outlined in the British Committee for Standards in Haematology best practice

Table III. Pathological investigative techniques in PTLD (after Harris *et al*, 2001).

Technique	Necessity	Purpose
Routine morphology	Essential	Architecture (preserved or effaced Cytology (polymorphic or monomorphic) Identification of specific morphological features (plasmacytoma, Burkitt lymphoma)
Immunophenotyping	Essential	Lineage Light chain restriction (flow cytometry or immunohistochemistry) Prognostic markers in some lymphomas
EBER ISH	Essential	Detection of EBV as an aid to diagnosis; PTLD versus rejection in allograft
Molecular genetic studies of antigen receptor genes	Useful	Clonality
Fluorescent *in situ* hybridization	Rarely required	Detection of specific translocations in some lymphomas
EBV clonality	Rarely required	Identification of minor clones

PTLD, post-transplant lymphoproliferative disease; EBV, Epstein-Barr virus; EBER ISH, EBV-encoded RNA *in situ* hybridization.

guidelines (Parker *et al*, 2007). Needle core biopsy and fine needle aspirate cytology may be useful in some cases (Gattuso *et al*, 1997; Siddiqui *et al*, 1997).

A full history should be provided with the specimen, and the pathological investigations include morphological assessment, immunohistochemistry and EBV staining, usually EBV-encoded RNA (EBER) *in situ* hybridization, which is more sensitive than immunohistochemistry (Harris *et al*, 1997). Molecular biology may be required to assess clonality and specific translocations can be detected using fluorescence *in situ* hybridization (FISH) Table III lists the techniques that are used in the pathological evaluation of PTLD (Harris *et al*, 2001).

Pathological interpretation of PTLD

The pathological diagnosis of PTLD is based around the World Health Organization (WHO) classification of these conditions, shown in Table IV (Swerdlow *et al*, 2008a).

There are four major WHO categories of PTLD – early lesions, polymorphic PTLD, monomorphic PTLD and classical Hodgkin lymphoma (CHL)-type PTLD. The monomorphic variety is further sub-categorized by lineage and according to the type of lymphoma present. Both lineages contain an 'other' categories to cover less common forms of PTLD, such as mucosa-associated lymphoid tissue (MALT)-type B-cell PTLD and NK cell PTLD (Kwong *et al*, 2000; Tsao *et al*, 2004). A Canadian multi-centre study reported an overall PTLD incidence of up to 14·6% in solid organ transplant recipients. Most cases showed nodal disease, and the majority were monoclonal. The lesions were of B-cell origin in 42·2% and of T-cell lineage in 15·6%. The PTLD was classified as monomorphic in 31·1%, polymorphic 18·9%, and hyperplastic in 1·1% (Allen, *et al* 2001).

Table IV. WHO categories of PTLD.

WHO Classification of PTLD
Early lesions
Plasmacytic hyperplasia
Infectious mononucleosis-like
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
T-cell Neoplasms
Peripheral T-cell lymphoma, not otherwise specified
Hepato-splenic lymphoma
Other
Classical Hodgkin lymphoma-type PTLD

In practice, a clear separation between the different WHO categories of PTLD is not always possible and early lesions, polymorphic PTLD and monomorphic PTLD probably represent a pathological spectrum (Nalesnik, 2001).

Early lesions

Early lesions are seen within a year of transplantation and are more common in transplant recipients with no previous EBV exposure. Two histological patterns are described – plasmacytic hyperplasia (PH) and IM-like. In PH lymphoid tissue shows sheets of plasma cells with scattered EBV-positive large immunoblasts. The underlying architecture of the lymphoid tissue is maintained and the plasma cells show polytypic light chain expression. In IM-like PTLD the histology resembles that

seen in IM. The architecture is at least partially preserved, and there is an expansion of the paracortical region by a mixed infiltrate composed of immunoblasts and plasma cells. The immunoblasts are present in much greater numbers than in PH, and consist of a mixture of EBV-infected B-cells (EBER positive) and reactive T-cells. The plasma cells that are present show polytypic light chain expression. These two early lesions show a morphological overlap; although IM-like PTLD contains a larger population of immunoblasts there is no clear cut-off point. Molecular studies show a polyclonal or oligoclonal IgH picture, and analysis of episomal EBV DNA shows that the virus is also polyclonal or oligoclonal.

Pitfalls and differential diagnosis. The histology of early PTLD, PH in particular, is not specific, so knowledge of a transplant history is vital. PH can mimic a plasma cell neoplasm, but there will be no evidence of clonality. IM-like PTLD with a high mitotic rate and focal necrosis can resemble an EBV-positive diffuse large B cell lymphoma (DLBCL) (d'Amore *et al*, 1996; Park *et al*, 2007). However the PTLD shows at least some preservation of tissue architecture, the immunoblasts present are a mixture of B and T cells, and the B cells are polyclonal. Occasional EBV-infected B-cells in IM-like PTLD can resemble Reed-Sternberg (RS) cells, and may be CD30 positive, but will be CD15 negative and show strong expression of CD20 (Reynolds *et al*, 1995).

Recommendations for the diagnosis of early PTLD lesions

Histology. **Preservation of underlying tissue architecture. PH shows plasma cells with scattered immunoblasts. IM-like lesions show predominantly immunoblasts, sometimes with RS like cells and/or plasmacytic differentiation. (Grade C level IV).**

Immunophenotype. **Plasma cells show polytypic light chain staining. Immunoblasts are CD20$^+$, CD79a$^+$, PAX-5$^+$, CD30$^+$, CD15$^-$. Admixed T-immunoblasts present (CD3+, CD5+). EBER positivity in B-immunoblasts. (Grade C level IV).**

Molecular analysis. **Oligoclonal or polyclonal IgH and T-cell receptor (TCR) genome. EBV genome oligoclonal or polyclonal. (Grade C level IV).**

Polymorphic PTLD

This form of PTLD is composed of a 'polymorphic' mixture of lymphoid cells that include small to intermediate-sized lymphocytes, immunoblasts and mature plasma cells. The infiltrate destroys the underlying tissue architecture, and may show malignant features – nuclear atypia, necrosis and a high mitotic rate. Immunohistochemistry shows a variable mixture of T cells and B cells. The B cells are EBER positive, CD30 positive and usually show monotypic light chain expression, although rare cases are polytypic. Molecular analysis demonstrates a clonal *IGH* rearrangement, even when light chains are polytypic, and the virus is also clonal (Kaplan *et al*, 1994).

Pitfalls and differential diagnosis. Effacement of the underlying tissue architecture is an important feature, and demonstration of clonality can be critical. Graft rejection can show a polymorphic lymphoid reaction, but the finding of EBV positivity in numerous cells indicates a PTLD. In some cases a single cell type (immunoblasts or plasmablasts) can predominate, producing a monomorphic appearance.

Recommendations for the diagnosis of polymorphic PTLD

Histology. **Effacement of underlying tissue architecture and a mixed infiltrate with small lymphocytes, intermediate-sized lymphocytes, immunoblasts and plasma cells. There may be necrosis, a high mitotic rate and nuclear atypia. (Grade C level IV).**

Immunophenotype. **B cell (CD20, CD79a, PAX-5) markers are positive and CD138 highlights plasma cells. The majority of B cells are EBER+. Light chains may be polytypic or monotypic. (Grade C level IV).**

Molecular analysis. **Clonal IGH genome (TCR is usually polyclonal). Clonal EBV genome. (Grade C level IV).**

Monomorphic B-cell PTLD

This is the most common form of PTLD and does not show the range of cell types seen in polymorphic PTLD. There are four main categories of monomorphic B cell PTLD:

Diffuse large B-cell lymphoma (DLBCL). The majority of monomorphic PTLD cases fall into the DLBCL category. The pathological features are those of DLBCL – the cells express B cell antigens (CD20, CD79a, and PAX5) and are EBV positive by EBER *in situ* hybridization. Monotypic immunoglobulin light chain expression is found in 50% of cases. Genetic studies show clonal *IGH* rearrangement in the majority of cases, and the EBV genetic material is also clonal (Kaplan *et al*, 1994).

Burkitt lymphoma (BL). This PTLD shows the morphology and phenotype of sporadic BL. The cells are monomorphic with rounded nuclei, small nucleoli and basophilic cytoplasm. There is a high level of apoptosis, and macrophages containing apoptotic debris can produce the typical 'starry sky' histological appearance. The cells show the characteristic Burkitt immunophenotype – CD20 positive, CD10 positive, BCL6 positive and BCL2 negative. There is widespread expression of EBER and Ki67 shows a proliferation fraction that is close to 100%. Genetic studies show a clonal *IGH*

rearrangement and a *MYC* translocation similar to that seen in BL in non-transplanted patients.

Plasma cell myeloma and plasmacytoma-like lesions. This rare form of monomorphic B-cell PTLD can present clinically as a solitary extramedullary plasmacytoma or as plasma cell myeloma (Sun *et al*, 2004; Caillard *et al*, 2006). There may be monoclonal immunoglobulin in the serum or urine. Histologically, the lesions are composed of mature plasma cells, with typical 'clock-face' or 'cartwheel' nuclear chromatin. CD20 and PAX5 are usually negative; CD79a expression may be maintained. Plasma cell markers CD138 and VS38c are positive, and light chain staining usually shows a monotypic picture. There is clonal *IGH* rearrangement, but staining for EBV may be either positive or negative (Sun *et al*, 2004).

Pitfalls and differential diagnosis. There is an overlap between DLBCL-PTLD and Burkitt-like PTLD; cytogenetic studies may be required to distinguish the two entities. Distinguishing plasmacytic DLBCL from a pleomorphic plasma cell neoplasm may be problematic, and whilst retention of CD20, CD19 and PAX5 can be helpful, some plasma cell neoplasms show aberrant CD20 expression (Cao *et al*, 2008). CD30 positivity in DLBCL-PTLD may suggest CHL or anaplastic large cell lymphoma (ALCL). The presence of EBV and B cell markers will exclude ALCL, and DLBCL is ALK1 and EMA negative. BL can resemble B lymphoblastic leukaemia/lymphoma or mantle cell lymphoma, but terminal deoxynucleotidyl transferase (TDT), CD5 and cyclin-D1 will be negative. The plasma cell forms of PTLD can be distinguished from reactive plasmacytosis by the detection of light chain restriction or clonality.

Recommendations for the diagnosis of monomorphic B-PTLD

Histology. **Destruction of underlying tissue architecture and malignant cytological features. DLBCL type shows immunoblastic, centroblastic or pleomorphic morphology. BL type shows monomorphic cells with prominent apoptosis ('starry sky' pattern). Plasmacytic lesions contain sheets of mature plasma cells. (Grade C level IV).**

Immunophenotype. **DLBCL B-PTLD is $CD20^+$, $CD79a^+$, and $PAX\text{-}5^+$. May be focally $CD30^+$. Light chain restriction in 50%. $EBER^+$.**

BL-PTLD is $CD20^+$, $CD10^+$, $BCL2^-$ with approaching 100% positivity using Ki67/MIB1. EBER+. Light chain restriction is common.

Plasma cell myeloma and plasmacytoma-like PTLD is $CD138^+$, $VS38c^+$, $CD20^-$ (usually), $CD79a^+$. Light chains are monotypic. EBER±. (Grade C level IV).

Molecular analysis. **Clonal IGH genome (TCR usually polyclonal). Clonal EBV genome if EBV positive. BL-PTLD shows *MYC* rearrangement. (Grade C level IV).**

Monomorphic T-cell PTLD

All T-cell PTLD are classed as monomorphic and this group includes T/NK cell lesions. The cases are classified using the WHO categories for T-cell lymphomas arising in non-transplanted patients (Swerdlow *et al*, 2008b). The morphology and phenotype are highly variable and any WHO type of T-cell lymphoma can be seen in the post-transplant setting. Immunophenotyping is essential for the diagnosis and requires both pan-T cell markers (CD2, CD3, CD5 and CD7) and T cell subset markers (CD4 and CD8). Pan-T cell markers are usually positive, but loss of one or more of these antigens is not infrequent. Most cases are CD4 positive and CD8 negative, but any combination of subset markers can be seen. Larger cells are often CD30 positive and ALCL-PTLD has been described (Pitman *et al*, 2004). Cytotoxic markers (perforin, granzyme and TIA-1) and CD56 are positive in some cases. The majority (60–90%) of T-PTLD are EBV negative (Dockrell *et al*, 1998). Molecular analysis of the TCR gene shows a clonal rearrangement.

T/NK PTLD is a rare condition and shows expression of CD56 and cytotoxic markers with negative CD3 staining. CD2 and CD7 staining can be positive but molecular analysis should show a germline TCR pattern. Most NK cell PTLD are EBV positive, with clonality of the EBV episomal DNA (Kwong *et al*, 2000; Tsao *et al*, 2004). Approximately 20% of hepatosplenic T cell lymphomas (HSTL) arise in the setting of chronic immunosuppression, most commonly following solid organ transplantation (Khan *et al*, 2001; Swerdlow *et al*, 2008a). Histological examination of spleen, liver or bone marrow show sinusoidal infiltrates of neoplastic T cells that are usually CD3 and CD2 positive and CD4 negative. In most cases the cells express the gamma-delta T cell receptor, but an alpha-beta variant is also recognised. There is clonal rearrangement of the TCR and frequent finding of isochromosome 7q. EBV is generally negative, but occasional cases are EBER positive (Khan *et al*, 2001).

Pitfalls and differential diagnosis. Cases showing partial involvement of a lymph node may be mistaken for reactive paracortical hyperplasia. Aberrant expression of CD20 or CD79a by neoplastic T-cells can lead to a mistaken diagnosis of B cell PTLD, and cases expressing CD30 and EBV can mimic CHL type PTLD. A clonal proliferation of T-large granular lymphocytes has been reported in solid organ transplants (Sabnani *et al*, 2006), but this is thought to be due to chronic antigen stimulus and not to represent a true PTLD.

Recommendations for the diagnosis of monomorphic T-PTLD

Histology. **Destruction of underlying tissue architecture and malignant cytology. A wide range of morphological appearances depending upon the type of T-cell lymphoma. (Grade C level IV).**

Immunophenotype. **Variable expression of pan-T antigens (CD3, CD5, CD2, CD7). Any combination of subset markers. Cytotoxic markers often positive. CD30 can be positive and aberrant CD20 expression can be seen. T/NK cases are CD56+, CD3-. Most (60–90%) T-cell cases are EBER-. T/NK cell lesions are usually EBER+. (Grade C level IV).**

Molecular analysis. **Clonal TCR genome (germline in T/NK lesions). IgH can be polyclonal or monoclonal. EBV genome clonal (if present). (Grade C level IV).**

Classical Hodgkin lymphoma-type PTLD

This rare form of PTLD shows the histology of CHL and is seen as a late complication of transplantation. Biopsy shows RS cells and variants on a mixed background of small lymphocytes, histiocytes and eosinophils. The RS cells show the typical phenotype; CD30 positive, CD15 positive, EBV positive, CD45 negative, and CD3 negative. CD20 may be negative or show weak and/or heterogeneous positivity.

Pitfalls and differential diagnosis. Hodgkin lymphoma-PTLD should be distinguished from polymorphic PTLD or IM-like PTLD with RS-like cells. The EBV-infected B cells in these conditions can show RS morphology, but will lack CD15 expression and show positive staining with CD20 and CD45. PTLD cases showing RS morphology but with strong expression of CD20 can resemble 'grey zone' lymphomas (Rüdiger *et al*, 1998) and should be classed as monomorphic B cell PTLD.

Recommendations for the diagnosis of Hodgkin and Hodgkin-like PTLD

Histology. **RS cells and/or Hodgkin cells on the typical CHL background. (Grade C level IV).**

Immunophenotype. **HL-PTLD shows classical phenotype – $CD30^+$, $CD15^+$, $CD45^-$, $CD20^-$, $CD3^-$. RS cells usually EBER+. (Grade C level IV).**

Molecular analysis. **IGH genome can be monoclonal or polyclonal (TCR usually polyclonal). EBV genome is clonal. (Grade C level IV).**

Other forms of PTLD

The commonest other form of PTLD is the extra-nodal marginal zone B-cell lymphoma of MALT type. These are pathologically identical to MALT lymphomas seen in non-transplanted patients (Wotherspoon *et al*, 1996). EBV is usually negative in this form of PTLD, and there is a strong association with *Helicobacter pylori* (Hsi *et al*, 2000), particularly when the disease involves the stomach. The lesion is made of up of monomorphic small cells with a centrocyte-like or monocytic appearance and there may be evidence of plasma cell differentiation. The immunophenotype is that of MALT lymphoma – the tumour cells are CD20 positive, CD5 negative, CD10 negative, IgM positive and IgD negative with light chain restriction. Molecular analysis shows a clonal *IGH* rearrangement. Hairy cell leukaemia has also been reported in the post-transplant setting (Tsao *et al*, 2006) and T-lymphoblastic lymphoma/leukaemia has also been described (Tsao & Hsi, 2007). Occasional patients show a combination of both B-cell and T-cell PTLD (Yin *et al*, 2005).

Pre-transplant management

Screening

Pre-transplant screening is routinely applied to determine whether the donor or recipient is infected with EBV. In this context serology offers the best approach rather than nucleic acid-based amplification methodologies. In children under 1 year of age it is usual to assume seronegativity, as maternal antibodies may give rise to a false positive antibody status.

T-Cell archiving

While T-cell adoptive immunotherapy for EBV has advanced substantially, the possibility of archiving seropositive recipient T-cells to use for expansion and subsequent re-infusion remains an option (Comoli *et al*, 2002, 2005). To date, this has not been routinely adopted but, given the relative ease at which peptide stimulation and subsequent selection of T-cell clones can be achieved, such approaches offer an attractive alternative to chemotherapy. An alternative is to use allogeneic partially human leucocyte antigen (HLA) matched cells (Haque *et al*, 2007).

Recommendations

- **Donor and recipients should be screened for prior exposure and infection with EBV using approved serological assays detecting antibodies to EBV VCA or EBNA-1 (Grade C, level 4).**
- **Children under 1 year of age, irrespective of the results of the antibody assay, should be regarded as seronegative for risk stratification and post-transplant surveillance (Grade C, level 4).**

Post-transplant surveillance

There is a direct correlation between EBV proliferation and progression to PTLD. A lack of use of a common viral load standard and different methodologies used by laboratories means that it is difficult to compare viral loads between

laboratories. It is not possible therefore to determine a clinically useful 'cut off value' for the diagnosis of EBV DNAemia and risk for PTLD.

Value of EBV polymerase chain reaction (PCR) in EBV DNAaemia and PTLD

The principle of routine EBV PCR monitoring is that it allows the early detection of EBV DNAemia and, potentially, the use of pre-emptive management algorithms to be initiated (eg reduction of immunosuppression) thus, hopefully, preventing progression to PTLD. The use of EBV PCR monitoring has become widespread in the haemopoietic stem cell transplant community, but is mostly used to determine risk of developing PTLD where clinical features may be non-specific and the disease can progress rapidly.

Paediatrics. Usually a high EBV viral load precedes the development of EBV-related PTLD. Lee *et al* (2005) demonstrated, in a prospective study of monitoring EBV viral load in 73 paediatric liver transplant patients, that the incidence of PTLD was reduced (from 16% to 2%) by pre-emptively reducing immunosuppression in individuals with high EBV viral load. In individuals with a persistently negative EBV PCR, serial monitoring has been shown to have a negative predictive value of 100%. Green *et al* (1998) monitored 30 paediatric intestinal transplant recipients on an intensive protocol. In 11/30 children had persistently negative EBV viral load and none developed PTLD .

Conflicting reports exist on the use of EBV PCR viral load as a monitoring tool for response of PTLD to therapy in the paediatric setting depending on centre and transplanted organ.

Adults. Although over 60 papers have been published since 2000 on EBV loads in adult patients with PTLD there remains a paucity of published data in adult transplant recipients on the prognostic value of EBV load monitoring, especially based on prospective studies. Whelass *et al* (2008) reported a retrospective single centre study of 296 lung transplantation patients between 1990 and 2008. One hundred and five patients had EBV DNA levels measured, primarily when screening seronegative recipients, to investigate new lung nodules, pyrexia or clinical deterioration of unknown aetiology. Thirteen patients developed PTLD, of these five had viral load data. Only one case of PTLD occurred in a patient who was seropositive pre-transplant. No patient who was EBV DNA negative developed PTLD but the sensitivity and specificity of the test was only 60% and 30%, respectively.

The Groningen group have reported their experience on the prognostic value of EBV DNA load in prospectively collected blood samples (Stevens *et al*, 2001). They concluded that a high EBV level identifies a high risk group, although they did not report the EBV DNA load in the entire cohort who did not develop PTLD and so positive and negative predictive values could not be determined. The same group reported their experience of EBV DNA monitoring as a marker of the degree of immunosuppression (Bakker *et al*, 2007). It was noted that the EBV load fell significantly in response to reduction of immunosuppression. The authors concluded that pre-emptive reduction of immunosuppression guided by EBV DNA load is a safe approach for the prevention of PTLD late after lung transplantation. The weakness of this study is that patients were several years post-transplant and the risk of PTLD was presumably low; it is difficult to know if EBV monitoring had any effect on the incidence of PTLD.

The surveillance data is therefore conflicting, possibly due to the differences in the patient populations studied with respect to age and EBV serostatus at the time of transplant. There is some evidence to support the use in a paediatric population, particularly where the liver and small bowel are affected. However, in adults there is little evidence to support the routine use of surveillance EBV PCR outside the allogeneic haemopoietic stem cell transplantation setting.

Primary EBV infection and reactivation

Patients who are EBV seronegative pre-transplant are at risk for developing primary IM infection and those who are EBV positive can reactivate the infection without developing PTLD. This usually presents with the classic clinical features of 'glandular fever' consisting of sore throat with fever, tonsillar enlargement with exudates and cervical lymphadenopathy and in some cases splenomegaly and hepatitis. The clinical suspicion should be confirmed by EBV serology showing IgM antibodies, computed tomography (CT) scan of chest, abdomen and pelvis should not show other areas of involvement and lactic dehydrogenase levels should be normal. It is impossible to tell early PTLD from IM based on histology alone, but there are some laboratory tests that can help. These include expression of major histocompatibility complex (MHC) Class II expression on cytotoxic T cells (Amlot *et al*, 1996; Rees *et al*, 1998), cytoplasmic gamma interferon (IFNγ) expression in response to autologous or HLA-matched EBV transformed B cells (LBCL) (Guppy *et al*, 2007) and EBV genome copy number (Stevens *et al*, 2005).

These patients should undergo frequent and careful monitoring with a low threshold for biopsy. Patients must be monitored for EBV seroconversion at presentation and then monthly (IgM to IgG against VCA) until the stable appearance of IgG EBNA-1. If the lymph node or tonsillar enlargement does not improve, or indeed worsens, biopsy should be carried out. Resolution of IM usually occurs by 1 month post-presentation, but can take several months in some cases with fluctuation in lymph node or tonsillar size. It is not warranted to carry out extensive investigations in patients who have a high EBV genome copy number and seroconversion who are asymptomatic, but progressively rising EBV genome copy numbers is an indication for more extensive investigation. Rarely, these patients can develop a haemophagocytic syndrome without evidence of PTLD. Reduction in

immunosuppression is often required and should be considered, particularly if there are concerns that resolution of clinical features are not occurring with rising EBV copy number suggestive of early PTLD.

Chronic high EBV viral load

The development of EBV disease and PTLD is usually accompanied by the detection of a high EBV load in peripheral blood. However, the significance of high EBV viral load status in clinically well post-transplant patients on long-term health is not known.

Two paediatric studies have been reported. Bingler *et al* (2008) reviewed the data of 71 paediatric patients who had serial EBV PCR monitoring since 1997. They defined 'chronic high-load carriers' as EBV viral load >16000 copies/ml (or >200 copies/10^5 peripheral blood mononuclear cells) on at least 50% of samples over a minimum period of 6 months after asymptomatic infection or complete resolution of symptomatic EBV infection or PTLD. In 20/71 patients met the above criteria for 'chronic high load carriers' – 8 with prior EBV positive PTLD, seven with prior symptomatic EBV infection and five with primary asymptomatic EBV infection. Nine of the 20 'chronic high-load carriers' developed late-onset PTLD.

Another study suggests that many children undergoing primary EBV infection following liver transplant will maintain chronically elevated EBV loads in the absence of clinical symptoms. To better understand this phenomenon, a retrospective review of the records of children undergoing liver transplant from 1997 to 2007 was carried out to identify chronic high EBV load carriers in this population (Green *et al*, 2009). A chronic high load state was defined by the presence of a high load for >50% of samples for greater than or equal to six months following either asymptomatic or complete clinical resolution of EBV disease/PTLD. A total of 35 chronic high load carriers were identified. Pre-transplant serologies were available for 29 of the 35; 22/29 (76%) were EBV negative prior to liver transplantation; eight of these 22 developed their chronic high load state at the time of their primary EBV infection. Fourteen of the 35 had EBV disease ($n = 7$) or PTLD ($n = 7$) prior to development of the chronic high load state. Only one of 35 chronic high load carriers developed PTLD or lymphoma while they were a high load carrier. In all, 23/35 resolved their chronic high load state without apparent sequelae while 11 children continue to be asymptomatic high load carriers.

Frequency of monitoring

If viral load monitoring is to be done no consensus exists regarding the frequency of testing EBV viral load in the post-transplant follow-up period. Different centres use different protocols depending on the organ transplanted. There needs to be intense monitoring in paediatric transplants in the first year pos-transplant as this is the most vulnerable time for the development of EBV infection and PTLD. Some have observed a viral doubling time of approximately 56 h and propose a weekly or twice weekly monitoring frequency (Stevens *et al*, 2001) comparable to the monitoring patterns adopted for cytomegalovirus post transplantation which has a doubling time of approximately 24 h (Emery *et al*, 1999).

Host cellular immune responses

Primary infection by EBV normally provokes a profound immune response in which there is an increase in $CD8^+$ activated cell markers, including a large expansion in EBV-specific CD8 T-cells and also changes in EBV-specific CD4 T-cell response (Strang & Rickinson, 1987). Monitoring of this response should provide dynamic information on the impact of EBV in immunosuppressed patients and possibly predict the onset of PTLD and help in management of PTLD.

Enzyme-linked immunosorbent spot (ELISPOT) technique. The IFNγ capture assay is the commonest assay used to detect EBV-specific immune response. This relies on the ability of T cells to secrete cytokine on activation and IFNγ secretion correlates with specific target cell killing. Yang *et al* (2000) measured the aggregate response to EBV virus and concluded that ELISPOT technique was useful in detecting low frequency responses. Smets *et al* (2002) reported their experience in 45 children undergoing liver transplantation. Seven developed PTLD and all had a low anti-EBV T cell response and high viral load. The ratio between viral load and specific cellular response demonstrated a 100% predictive value regarding the emergence of subsequent PTLD.

HLA tetramers specific for EBV T cells. Viral-derived peptides are expressed on the surface of EBV-transformed lymphocytes, which facilitate the control of latent and lytic infection in healthy EBV-infected individuals. MHC class 1 tetramers have been developed to identify these antigen-specific T lymphocytes. Quantitative studies using MHC class I tetramers complexed with peptides derived from latent and lytic cycle EBV proteins have been used to quantify the cell specific immune response to EBV (Macedo *et al*, 2005). The major drawback at present is that these tetramers are HLA-specific and are most frequently available for HLA A2 patients.

Peripheral lymphocyte subsets. The importance of cytotoxic T cell response ($CD8^+$) in the host; in response to EBV infection has been well characterised. Following EBV infection, a strong HLA class I-restricted virus-specific $CD8^+$ CTL response is seen, followed by a resting latent state in the B cell population (see above).

Recent studies have demonstrated that NK cells may play an important role in the treatment and protection of EBV-associated lymphoproliferative disorder. Thirty four to 54% of

$CD8^+/HLADR^+$ and 34–60% of $CD8^+/CD45RO^+$ T cells in the peripheral blood mononuclear cells of patients suffering from IM have been shown to be EBV-specific. Monitoring of the lymphocyte response by flow cytometric techniques in EBV viraemia and EBV-driven PTLD can provide dynamic information on the impact of EBV in immune suppressed patients and possibly help in the management of PTLD. The fact that adoptive immunotherapeutic approaches often work best when a significant CD4 T-helper response against EBV is also present argues for the importance of these cells in the control of EBV replication via their provision of help to EBV-specific CD8 cytotoxic T-cells (Haque *et al*, 2007).

Recommendations

- **The routine surveillance of adult transplant populations for EBV DNAemia by PCR is not recommended outside the stem cell transplant population (Grade B, level 3).**
- **In children at risk of primary EBV infection, routine surveillance is likely to aid in pre-emptive identification of patients at high risk of PTLD (Grade B, level 3).**
- **The use of EBV DNA load measurement after initiation of therapy for PTLD to monitor response to therapy is not recommended (Grade B, level 3).**

Risk factors for PTLD

Immunosuppressive agents

The major modifiable risk factor for PTLD in solid organ transplantation is the degree of immunosuppression. However, there is no consensus as to whether any one particular immunosuppressive agent is particularly responsible for PTLD, or even if the overall degree or intensity of immunosuppression is directly linked to an increased risk of PTLD. The number of different immunosuppressive agents used following solid organ transplantation has increased in the last 20 years and almost all recipients receive more than one drug. In addition, supportive care has also improved over this time period with increasing numbers of patients having long term survival. It is, therefore, difficult to separate the influence of any one particular agent on the development of PTLD, and the risk of rejection of the transplanted organ if reduced immunosuppression is used must also be considered by the clinician.

Ciclosporin. PTLD was rare prior to the introduction of ciclosporin and a dramatic increase in incidence of PTLD was seen during the early experience of this agent. This was greatly reduced once drug-level monitoring became widespread and doses were subsequently reduced (Beveridge *et al*, 1984; Starzl *et al*, 1984).

Two large single-centre studies have reported their experience of PTLD over many decades. The Stanford group have reported 30 years experience of PTLD following heart and heart/lung transplantation. They divided recipients into four eras. The first were transplanted prior to the introduction of ciclosporin, the second group received ciclosporin, azathioprine and prednisone and the third group received this agent plus a monoclonal antibody (OKT3), a murine anti CD3 antibody, which is profoundly T cell depleting. The fourth group received agents such as tacrolimus instead of ciclosporin, and mycophenolate (MMF) substituted for azathioprine. Interestingly, there was no significant difference in the incidence of PTLD between the four groups despite the different immunosuppressive agents. There are several possible explanations for this result, which is in contrast with the experience of the majority of centres. Probably the most likely reason is that of competing risks (principally for death from transplant failure), which reduces the likelihood of the analysis having enough patient years at risk to rigorously test the influence of the different drug therapies. When the patients receiving ciclosporin were sub-analysed, according to the dose received, the incidence of PTLD was 10% in the high dose ciclosporin group, which was approximately twice the rate of the lower dose group (Gao *et al*, 2002).

In another single centre analysis, from Oxford, data was presented from 1500 renal transplant recipients over a 23-year period from 1976. Three immunosuppressive eras were defined. The first: azathioprine and prednisone, the second ciclosporin, azathioprine and prednisone, and then newer agents including tacrolimus and MMF. In this series one case of PTLD was seen prior to the introduction of ciclosporin, after which the incidence rose to 0·8% and then 2·3% in the most recent era. In common with many reports, this can only give an indication as to the incidence, given that the years at risk will be very different in the different groups (Libertiny *et al*, 2001).

Leuco-depleting antibodies. The Spanish Heart Transplant Registry analysed data from 3393 patients (transplanted between 1984 and December 2003) who survived at least three months after heart transplantation (Crespo-Leiro *et al*, 2007). The patients were divided into three eras: 1984–1997 (pre-MMF, 1810 patients), 1998–2000 (between the introduction of MMF and Interleukin 2 Receptor (IL2R) blockers, 844 patients) and 2001–2003 (748 patients). Induction therapy was used in 60·5% of patients; 44% received OKT3, 17% received Anti -Thymocyte Globulin (ATG), 11% received IL2R blockers. Eight four percent of patients received ciclosporin at some stage and 22% were treated with tacrolimus. There was no difference in PTLD incidence in any era. No immunosuppressive regime, except ATG, was associated with a change in PTLD incidence. In patients who did not receive prophylactic aciclovir or ganciclovir, ATG and OKT3 were both associated with an increased risk of PTLD. The authors speculated that the dose of OKT3 used in these patients was lower than that in other reports accounting for the lack of association with PTLD. They did not report the dose of ATG (Crespo-Leiro *et al*, 2007).

In a registry of 50 000 renal and heart transplant recipients, the incidence of PTLD was approximately three-fold higher in patients receiving T cell depleting antibodies [OKT3, ATG and Anti-Lymphocyte Globulin (ALG)] (Opelz & Henderson, 1993). This is probably the most compelling data that quantifies the increased risk of PTLD secondary to T cell depleting antibodies. In recent practice, in many solid organs the T and B cell depleting anti-CD52 antibody, alemtuzumab, has been used, often followed by reduced doses of maintenance immunosuppressants. Single centre experience of 297 patients, who received this antibody as part of an induction protocol, and were followed for between 2 and 4 years, recorded no cases of PTLD (Ortiz *et al*, 2008). There have only been three randomized controlled trials of the antibody published and longer term follow up will be necessary to decide if it has an improved risk profile when compared with other leucocyte depleting antibodies (Ciancio *et al*, 2008).

Tacrolimus. Tacrolimus has a similar mode of action to ciclosporin and early reports of its use in children with liver transplants showed PTLD rates of approximately 20% in comparison to around 3% previously reported from at least one of these centres (Reding *et al*, 1994; Cox *et al*, 1995). Although these very high rates may have been because of inappropriate dosing, more recent reports, including paediatric and adult solid organ transplantation experience, suggests tacrolimus use instead of ciclosporin is associated with an approximately two- to five-fold increase in the risk of developing PTLD (Cao *et al*, 1999; Younes *et al*, 2000; Opelz & Döhler, 2004).

Mycophenolate mofetil. Interpreting data about any influence of MMF on the risk of PTLD development has the usual problem that the agent is always used in combination with other drugs and, in recent experience, most commonly in conjunction with tacrolimus. There are three large registry analyses that suggest MMF use is associated with either a reduced or no change in the rate of PTLD. In the North American paediatric renal database, 108 cases of PTLD are recorded in 6720 transplants. The use of MMF was not associated with any increase in rate of PTLD and, in the more recent era, no cases of PTLD are reported with the combination of tacrolimus and MMF, although this observation must be treated with caution given the differential length of follow up between the two groups (Dharnidharka *et al*, 2002). From two large renal transplant registries, 6751 patients identified as receiving MMF and an equal number of controls were followed for 3 years. There was no significant difference in the risk of PTLD with a trend towards a reduced risk (Robson *et al*, 2005). From the International Heart and Heart / Lung Transplant Registry the results of 3895 transplants were analysed. Although the authors stated, when comparing patients treated with MMF to those who were not: 'For the sub-group with PTLD/lymphoma, the risk was again found to be marginally significant (adjusted RR = 0·44, 95% confidence interval 0·19–1·01, $P = 0·054$)' this risk reduction was not, in fact, by conventional statistical measures, significant and the most appropriate interpretation is that MMF has not been shown to be associated with any alteration in the risk of PTLD (O'Neill *et al*, 2006).

Mammalian target of rapamycin (mTOR) inhibitors. Rapamycin, sirolimus and everolimus are inhibitors of the mTOR signalling pathway and are used in a variety of solid organ transplant protocols. In patient-derived PTLD tissue samples, activation of the mTOR signalling pathway was found in the entire spectrum of PTLD subtypes (El-Salem *et al*, 2007). This laboratory study suggests that mTOR inhibitors may be effective in treatment and, by extension, prophylaxis against PTLD. A number of single centres have reported low levels of PTLD in sirolimus-treated patients (Jaber *et al*, 2007). The largest experience is of 349 renal transplant recipients, with at least 3 years follow-up, who had a complicated regimen, but were maintained on a calcineurin inhibitor and then either MMF or sirolimus. In this regimen, no PTLD had been reported at the time of publication (Khwaja *et al*, 2004).

Interleukin-2 receptor blocking antibodies. The non-leucodepleting IL2R blocking antibodies, basiliximab and dacluzimab, have been part of many prophylactic immunosuppressive therapy regimens for several years. In a single centre report, in a relatively high risk group, 54 paediatric liver transplant recipients were treated with basliximab, prednisone and ciclosporin and followed for between 22 and 46 months. There were no cases of PTLD compared with one case in 54 historical controls treated with only ciclosporin and prednisone (Ganschow *et al*, 2005). In a review of the experience of basiliximab post renal transplantation with follow up of a maximum 5 years there was no increased risk of PTLD seen compared with placebo (Chapman & Keating, 2003).

Age

PTLD is commonest in children under 10 years and adults over 60 years when compared with the normal population, being highest in paediatric small bowel transplant recipients and lowest in adult liver transplant recipients (see Table V). The increased incidence in children and adolescents is seen within the first 3 months of transplant and is primarily thought to be due to primary EBV infection (Smith *et al*, 2007). The relative risk decreases as the incidence of lymphoma rises with age in the general population, such that the relative risk for PTLD in a cardiac transplant patients under 10 years of age is 1240·2 (95% confidence interval (CI) 814–1892) which reduces to 16·4 (95% CI 13–21) in patients over 60 years old (Opelz & Döhler, 2004).

Table V. Approximate frequency of PTLD in patients by organ transplanted & age.

Organ	Adults (%)	Paediatric (%)
Kidney	1·0–2·3	1·2–10·1
Liver	1·0–2·8	4·0–15·0
Heart	1·0–6·3	6·4–19·5
Heart/Lung	2·4–5·8	6·4–19·5
Lung	4·2–10·0	6·4–19·5
Small bowel	20	30

Length of time post transplant

The length of time from the organ transplant date does appear to influence the risk of developing PTLD. If the cumulative risk is assessed there is an increase, with one series of 31 patients having 10% in the first 2 years, 30% in the next 3 years and then 60% occurring more than 5 years post transplant (Dotti *et al*, 2002). However, this may be influenced by the different immunosuppressive regimens used with the various cohorts. In the paediatric setting there is a high incidence in the first 3 months post transplant thought to be due to primary infection with EBV (Katz *et al*, 2007). A recent analysis by UNOS for PTLD in USA transplant recipients 2003–2007 found the rate of PTLD at 3 years post-transplant on both adult and paediatric recipients to be lowest for kidney recipients and highest for intestine recipients (Ison & Nalesnik, 2009).

Ethnic origin

Ethnic origin may be a factor; a study from the USA showed that the rate in Caucasians was double that of those of African descent (Smith *et al*, 2006).

Conclusion

It is difficult to calculate the relative risk of PTLD for any one individual. The risks of PTLD vary with the solid organ that is transplanted and whether the recipient is an adult or a child. These are clearly not modifiable risk factors. Although the risk of PTLD was very low in patients treated with prednisone and azathioprine alone this is not an appropriate reference point, as the risk of graft failure with that regimen is so high that it is not sensible to consider it further as an option. Of the induction agents, IL2R antagonists do not seem to increase PTLD risk. The data on OKT3 and various forms of ATG are difficult to interpret because the dose and duration of therapy varies so much; however both probably increase the risk of PTLD.

Most papers are reports of registry data and different maintenance drug regimens have been used in different time periods. It is, therefore, difficult to demonstrate a convincing association between any particular maintenance immunosuppressive drug and PTLD, except perhaps a decrease in risk with mTOR inhibitors. There appears to be some evidence of an increased risk with tacrolimus compared to ciclosporin. MMF does not appear to confer an increased risk.

When considering the possibility of PTLD as part of a differential diagnosis, it is important to consider each individual patient's risk factors for developing PTLD.

Recommendation

- **The potential risk of PTLD should not influence the choice of immunosuppressant regimen (Grade B, Level 3).**

Clinical features and diagnosis

Clinical features

The clinical features of PTLD are often non-specific. The disease is commonly extra-nodal and, therefore, the classic feature of lymphadenopathy is frequently absent. Presenting features are more often related to interference with the function of the system that is involved. However, the classic B symptoms of weight loss, sweats and pyrexia can still occur, but may more often be found in association with symptoms related to the organ involved rather than lymphadenopathy (Dotti *et al*, 2002; Dhillon *et al*, 2007). It should also be remembered that bone marrow involvement can occur without any other system involvement so that the only feature may be a fall in peripheral blood counts.

Organ involvement

The classical presentation seen in the non-transplant population of enlarging lymph nodes as the sole feature is relatively uncommon (<10%) in PTLD, irrespective of transplant type, with extra nodal organ involvement being seen most commonly (Bates *et al*, 2003). However, the presence of a rapidly enlarging lymph node in an at risk patient should be investigated promptly, as should the development of enlarging tonsils/ adenoids, which should be investigated with biopsy, as these are frequently sites of PTLD, particularly in children (Posey *et al*, 1999; Broughton *et al*, 2000).

Transplant type

Analysis of over 200 000 patients in the collaborative transplant study database identified PTLD involving the transplanted organ in heart, lung or liver transplants in 52% of cases whereas, in renal transplant recipients, the commonest site was the gastrointestinal tract at 15·3% (Opelz & Döhler, 2004). Central nervous system (CNS) involvement was reported in up to 30% of patients with PTLD (Penn & Porat, 1995; Boubenider *et al*, 1997; Dror *et al*, 1999) although it may

well be reducing with alteration in immunosuppressive regimens (Robson *et al*, 2005). More recently, the commonest transplanted organ associated with the development of CNS disease was the kidney at 11·9% whereas in other organs it was <5% (Bronster *et al*, 2000; Buell *et al*, 2005). For patients who have had a liver transplant following liver failure due to underlying hepatitis C infection the risk of PTLD appears to be increased irrespective of the type of immunosuppression used (Hézode *et al*, 1999). A recent analysis by UNOS for PTLD in USA transplant recipients between 2003 and 2007 found the rate of PTLD at 3 years post-transplant in both adult and paediatric recipients to be lowest for kidney recipients and highest for intestine recipients (Ison & Nalesnik, 2009).

Techniques for diagnosis

Biopsy

The diagnosis of PTLD should be based on histological examination of tumour tissue obtained by surgical excision biopsy or needle core biopsy. A surgical excision tissue biopsy has the advantage of ensuring that sufficient tissue for full histopathological evaluation is obtained. A needle core biopsy may be preferred as a less invasive alternative, but multiple cores may be needed to provide adequate tissue for full analysis. The choice between open and needle biopsy and the approach to tissue biopsy depends on the anatomical site, size and distribution of suspected PTLD.

When intrathoracic PTLD is suspected, CT scan-guided transthoracic needle biopsy of pulmonary nodules or enlarged mediastinal lymph nodes may be used to provide tissue for diagnosis (Halkos *et al*, 2004). Open lung biopsy has been shown to be a useful diagnostic tool in helping to differentiate PTLD from other causes of suspicious pulmonary nodules with a relatively low complication rate (Choong *et al*, 2006). Gastric MALT lymphoma with associated *H pylori* infection has been described in transplant recipients and should be considered in individuals who develop symptoms of peptic ulcer disease (Aull *et al*, 2003). In recipients with intestinal transplants, chronic ulceration should be considered as suspicious and biopsies taken, not only from the ulcer edge, but also from the intervening mucosa (Selvaggi *et al*, 2006). Laparoscopic lymph node biopsy has been shown to be a safe and effective alternative to laparotomy in patients with intra-abdominal lymphoma and associated lymphadenopathy (Casaccia *et al*, 2007). Finally, endoscopic biopsy of gastric and colonic lesions may provide sufficient tissue for diagnosis of PTLD, although for small bowel PTLD laparotomy and excision biopsy is often necessary.

In some cases the sole site of involvement may be bone marrow or CNS. A bone marrow aspirate and biopsy should be considered in any patient with falling peripheral blood counts for whom obvious causes, such as drugs, infection etc., have been excluded. It should also be considered as part of the staging process of the lymphoma. For patients with suspected CNS disease a lumbar puncture with cerebrospinal fluid sent for immunophenotyping by flow cytometry as well as cytology may be helpful if meningeal disease is present, but in many cases biopsy or fine needle aspirate may be the only possibility to obtain tissue.

Imaging

There are no large studies comparing the suitability of different imaging techniques in PTLD and the published work consists of case series or individual case reports. Much of the relative importance of the different imaging techniques available, therefore, needs to be inferred from work done within the field of non-transplant related lymphoma diagnosis and staging in general and adapted to the particular circumstances of the post transplant patient (Seam *et al*, 2007; Kwee *et al*, 2008).

Ultrasound imaging. Ultrasound (USS) is useful at picking up changes in solid organs, such as liver and kidney grafts, and can detect the development of changes due to PTLD within the abdomen in some cases. Routine ultrasound of the abdomen in a paediatric transplant population detected 6/7 patients with intra-abdominal disease (Riebel *et al*, 2007). However, it was less useful in assessing renal transplant abnormalities in 24 patients with only 25% of abnormalities being correctly diagnosed and a high incidence of false positive masses (Ali *et al*, 1999).

Computerised tomography (CT). The role of CT scanning in PTLD can be helpful in identifying areas for biopsy, staging and treatment response. The diagnosis of PTLD can be difficult due to the rarity of the condition and the myriad sites of presentation, therefore, the possibility of PTLD must always be considered. In one centre, a review of abdominal CT scans carried out for obstruction post liver transplant showed PTLD to be an uncommon cause, found in only 3 of 44 patients (Blachar & Federle, 2001). Pulmonary CT findings need to be interpreted carefully, taking into consideration the patient's clinical state, particularly where extra-nodal disease is suspected, to ensure that all other differential diagnoses eg. aspergillus, biopsy scar, drugs etc have been excluded, because ground glass changes, single nodules and effusions can all be found in these conditions as well as PTLD (Collins *et al*, 1998; Copp *et al*, 2006).

CT imaging is not just important for making an initial diagnosis, but also for assessing the extent of the disease. The role of staging abdominal CT scans was shown to be of benefit in a single centre series of 51 patients with PTLD. All patients had a staging CT with approx 50% of the initial diagnosis being from an extra-abdominal site. Twenty nine percent of patients had no evidence of abdominal involvement by CT, of the remaining 70%, 22% had nodal involvement and the remainder had extra-nodal involvement with liver commonest (53%) and spleen and small bowel next commonest. In addition, this group observed that over 90% of liver and heart transplants had evidence of intra-abdominal involvement as against only 50% of renal and lung recipients (Pickhardt & Siegel, 1999).

The role of CT in initial diagnosis and staging of lymphoma has long been accepted, although it has a number of problems. In particular, it is neither able to identify involved nodes that have not increased in size nor to distinguish enlarged nodes due to non-malignant causes from malignancy. In addition, CT is poor at identifying bone involvement, which would automatically put a patient into Stage IV disease. However, when used with contrast, where appropriate, it provides valuable information about the size and location of tissues involved with lymphoma and has become one of the major tools for lymphoma diagnosis and staging.

^{18}F-fluoro-2-deoxyglucose positron emission tomography (FDG-PET) and FDG-PET/CT. The FDG-PET scan shows areas of increased metabolic activity and has advantages over CT in that it can identify areas infiltrated by lymphoma that have not yet increased in size, and is better at detecting bone involvement (Newman *et al*, 1994; Carr *et al*, 1998). It is poor at localising the precise area of involvement unless combined with CT (Marom *et al*, 2004; von Schulthess *et al*, 2006), but has a higher positive predictive value than CT alone (Seam *et al*, 2007). However, lymphomatous infiltration cannot be distinguished from any other cause of increased isotope uptake due to glycolysis, such as infection, inflammation, granulomatous involvement and bone marrow recovering from chemotherapy (Juweid & Cheson, 2006). In addition, not all types of lymphoma are FDG-positive with reports of PET-negative disease in some T cell non-Hodgkin lymphoma and low-grade B lymphomas. The precise role of FDG-PET, even in the diagnosis and management of non-transplant related lymphoma, remains controversial, apart from Hodgkin lymphoma where it is becoming accepted practice (Juweid *et al*, 2007).

The published literature reporting the use of FDG-PET in PTLD diagnosis is limited. The prime role would appear to be in assessing response to therapy rather than in aiding diagnosis (Reams *et al*, 2003; O'Conner & Franc, 2005; Bakker *et al*, 2006; McCormack *et al*, 2006; von Falck *et al*, 2007).

Magnetic resonance imaging (MR). The place of MR in the diagnosis/ staging and ongoing management of non-transplant related lymphoma remains undetermined, although it can be extremely helpful in the diagnosis of bone involvement and CNS lymphoma (Castellano-Sanchez *et al*, 2004; Brennan *et al*, 2005; Kwee *et al*, 2008).

Recommendations

- **A tissue diagnosis is required (Grade C, level 4).**
- **CT scan of chest, abdomen and pelvis is essential for staging purposes (Grade B, level 3).**

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