Post-transplant lymphoproliferative disease (PTLD) after haematopoietic stem cell transplantation is a life-threatening complication and the number of patients at risk is increasing over time. In the majority of patients Epstein-Barr virus-related PTLD (EBV-PTLD) is diagnosed with a histological picture of B-cell lymphoma. In 2009 recommendations for diagnosis, monitoring and treatment of patients with EBV-PTLD were published. Definitions of preemptive treatment and therapy of probable or proven PTLD were introduced. Results of preemptive treatment and therapy of documented PTLD are discussed in this paper, with the emphasis on monoclonal antibody anti-CD20 rituximab effectiveness and indications for its applications. A comprehensive review of reported cases of PTLD and summary of results indicate that rituximab in monotherapy or in combination treatment is successful in 63% of confirmed PTLD cases and in 89% when used preemptively. Still, an analysis of factors determining the response to therapy is lacking.

Key words: Epstein-Barr virus, post-transplant lymphoproliferative disease, haematopoietic stem cell transplantation.

Rituximab in treatment of post-transplant lymphoproliferative disease after allogeneic stem cell transplantation

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Post-transplant lymphoproliferative disease (PTLD) represents a heterogeneous group of neoplastic lymphoproliferations, observed after haematopoietic stem cell transplantation (HSCT) or among recipients of solid organ transplants (SOT). Post-transplant lymphoproliferative disease development is related to iatrogenic suppression of T lymphocytes, caused by immunosuppressive therapy [1, 2].

Post-transplant lymphoproliferative disease tends to present in the first year after transplant, but may occur late, even many years after the procedure. The majority of early cases are associated with EBV (Epstein-Barr virus) infection and appear as B-cell proliferation. Up to 30% of PTLDs are EBV-negative and usually occur late after transplant. The aetiology of EBV-negative PTLD is unknown; however, an association with HHV8 (human herpes virus 8) has been described. The late form of PTLD and EBV-negative PTLD may appear as T/NK cell proliferation.

Epstein-Barr virus is a widespread human herpesvirus and more than 90% of the healthy population is seropositive. EBV leads to transformation and proliferation of infected B lymphocytes, but in healthy individuals this process is controlled by the immune system [3, 4]. Involvement of EBV in oncogenesis is proved in many malignancies, especially in lymphoproliferative diseases, both primary and secondary [5, 6].

Epidemiology of Epstein-Barr virus-associated post-transplant lymphoproliferative disease after haematopoietic stem cell transplantation

Post-transplant lymphoproliferative disease developing after HSCT is usually related to EBV reactivation or, less often, to primary infection. The varying incidence ranging from 0.45% to 29% of reported EBV-PTLD is related to intensity of immunosuppression used and reflects different practice between centres with respect to patient population, conditioning, graft versus host disease (GVHD) prophylaxis, and source of stem cells. Identified risk factors include HSCT from unrelated, mismatched or haploidentical donors, umbilical cord blood transplants, T-depletion (*in vivo* or *in vitro*), use of antithymocyte globulin (ATG), and also splenectomy, serological mismatch between donor and recipient and chronic GVHD (Table 1) [7, 8]. The risk of PTLD increases with two or more risk factors.

Table 1. Risk factors for EBV-PTLD development after alloHSCT

Major	Minor
unrelated/mismatch HSCT	primary EBV infection
T-cell depletion (*in vivo* or *in vitro*)	splenectomy
EBV serology mismatch	chronic GVHD
cord blood HSCT	

HSCT – haematopoietic stem cell transplantation; GVHD – graft versus host disease; EBV – Epstein-Barr virus

Today with increased use of transplants from alternative donors, populations at risk of EBV-related PTLD are growing over time. Early diagnosis and treatment is crucial to overcome poor prognosis of this condition [4, 7]. Recommendations for management of HSCT recipients at risk of PTLD were published in 2009 as part of ECIL (European Conference on Infections in Leukemia) guidelines [9].

Clinical presentation of post-transplant lymphoproliferative disease

The clinical picture of PTLD is variable and correlates with the type of transplant. HSCT recipients usually present with widespread and progressing disease, involving nodal and extranodal sites [10]. It is said that multiple sites of disease with involvement of the liver, gastrointestinal tract, lungs, central nervous system and/or bone marrow, influence the outcome of PTLD. However, there is no acceptable risk score in this setting. In the course of PTLD non-specific mononucleosis-like symptoms may be seen, such as fever, hepatitis, encephalitis or enteritis.

Localized forms of PTLD are diagnosed rarely. Reduction in immunosuppression leads to resolution in most of such cases. In solid organ recipients PTLD frequently involves the allograft [11]. Since rejection and infection may result in a similar clinical picture the diagnosis and appropriate treatment are very difficult and confusing.

Histology of post-transplant lymphoproliferative disease

The World Health Organization (WHO) classification of PTLD includes several forms: early benign lesions, polymorphic PTLD, monomorphic PTLD and classical Hodgkin lymphoma type PTLD [10].

The early PTLD lesions are defined as polyclonal B cell, T cell and plasma cell proliferation without phenotypic aberrancy. Infiltration, with architectural preservation, involves lymph nodes or tonsils and often regresses spontaneously or with reduction in immunosuppression. The early lesions are usually seen in children and young adults after solid organ transplants. In some cases, polymorphic or monomorphic PTLD may follow early lesions.

Polymorphic PTLD involves lymph nodes or extranodal tissue with architectural effacement. Immunophenotypic studies show the full range of B cell maturation from immunoblasts to plasma cells. Polymorphic PTLD occurs most commonly in children. Reduction in immunosuppression leads to regression in a variable proportion of cases.

Monomorphic PTLD fulfils the criteria for B-cell or T/NK-cell lymphoma. Diffuse large B-cell lymphoma (DLBC), Burkitt lymphoma and plasmacytoma are the most commonly recognized. Small B-cell lymphoma as follicular lymphoma or mucosa-associated lymphoid tissue (MALT) lymphoma are not designated as PTLD, even when arising in the post-transplant setting. T/NK-cell lymphomas constitute about 15% of PTLDs and include peripheral T-cell lymphoma, hepatosplenic T-cell lymphoma, anaplastic large cell lymphoma, T-cell large granular lymphocyte lymphoma and others [12].

Classical Hodgkin lymphoma is the least common major form of PTLD. The disease occurs more often among solid organ recipients than after HSCT.

Diagnosis of Epstein-Barr virus-associated post-transplant lymphoproliferative disease

According to the published definition, EBV-PTLD includes the following forms: proven EBV-PTLD, probable EBV-PTLD and EBV-DNAaemia [9].

Diagnosis of proven EBV-PTLD requires histological biopsy of the affected organ and confirmation of tissue architecture disruption by monoclonal lymphoid cell infiltration and detection of EBV gene products or EBV-encoded RNAs [5]. Most PTLDs are of B-cell origin with a picture of DLBC lymphoma or Burkitt lymphoma [12].

Probable EBV-PTLD is defined as significant lymphadenopathy (or other end-organ disease) with high EBV load in the blood, in the absence of other aetiological factors or established disease in high risk patients [5].

EBV-DNAaemia is related to detection of EBV-DNA in the blood. The correlation between the number of EBV-DNA copies and PTLD development has been described [13]. Regular monitoring of EBV load allows early, preemptive treatment to be started in patients at risk for PTLD development. The EBMT (European Group for Blood and Marrow Transplantation) survey shows that once weekly monitoring of EBV-DNA for 3-6 months after transplant is performed by more than 70% of European transplant centres [14]. Quantitative PCR to measure EBV-DNA load in whole blood is the recommended method in the posttransplant setting [9].

Management of Epstein-Barr virus-associated post-transplant lymphoproliferative disease

Management of patients with EBV-PTLD includes prophylaxis, preemptive treatment and therapy of probable or proven disease. Various methods and drugs are used for the treatment of PTLD: rituximab, reduction in immunosuppression, donor lymphocyte infusion (DLI), EBV-specific cytotoxic T lymphocyte (EBV-CTL), chemotherapy, surgery and antiviral agents. Among them, monoclonal antibody anti-CD20 rituximab is known to significantly improve the outcome of patients with post-transplant lymphoproliferative disease.

Rituximab

Rituximab, a genetically engineered chimeric monoclonal antibody, has the ability to deplete normal and malignant B lymphocytes expressing the CD20 antigen on their surface [15]. Efficacy of rituximab in treatment of non-Hodgkin lymphomas in monotherapy or in combination with chemotherapy has been confirmed in *in vitro* studies and clinical trials [16]. The drug significantly improves the rate of complete remission and overall survival of patients with B-cell lymphomas [17-19]. Currently it is the most commonly used monoclonal antibody in the clinical setting. Resistance to rituximab, either primary or secondary, is seen in about 50-60% of treated patients. The resistance pathways and its mechanism remain uncertain, but it may be mediated

by alteration in CD20 expression or signalling, FcγR polymorphism, overexpression of CD55 or CD59, or elevated apoptotic threshold [20].

Rituximab selectively depletes B cells in blood and lymph nodes and the degree of depletion correlates with response to therapy. Recovery of B cells usually starts 6-9 months after rituximab therapy. However, a majority of peripheral lymphocytes are immunologically immature even after 12 months. Decreased levels of immunoglobulins IgG and IgM are seen up to 5-11 months. Delay in B cell reconstitution and function may lead to infectious complications – an increased number of hepatitis B reactivations and progressive multifocal encephalopathy caused by polyomavirus JC have recently been documented [21, 22].

Rituximab is well tolerated with mild to moderate flu-like symptoms during infusion in most patients. Severe complications with hypotension, bronchospasm, arrhythmia, and multiorgan failure are observed in less than 10% of cases [20].

Therapy of probable and proven Epstein-Barr virus-associated post-transplant lymphoproliferative disease after haematopoietic stem cell transplantation

Rituximab is currently most often used in therapy of PTLD developing after HSCT. A comprehensive review of all published cases of PTLD treated with rituximab has shown its efficacy in 63% of cases, when used in monotherapy or in combination with other treatment modalities [4]. However, available data on PTLD therapy are based on studies of a number of case series [23]. A multicentre European analysis on rituximab efficacy in therapy of PTLD is ongoing. This study will hopefully define the prognostic factors for therapy outcome. Currently, the use of intravenous rituximab at a weekly dose of 375 mg/m^2 is recommended as first-line therapy (ECIL recommendation AII) [9]. Usually, clinical improvement and reduction or clearance of EBV-DNAaemia occurs within 4-8 weeks, and the early response to rituximab therapy might be of prognostic value [4]. Patients with resistance to rituximab or disease progression might be candidates for chemotherapy (recommendation CIII), although the data and experience on the use of chemotherapy in PTLD patients after HSCT are limited, with respect to regimen, dosage and timing [4].

All patients diagnosed with PTLD should be considered for reduction in immunosuppression therapy, whenever possible (recommendation BII). This approach is routinely recommended after solid organ transplants. However, in patients after HSCT it gives an improvement in 50% of patients, but has no influence on overall survival [4]. Another valuable treatment modality is immunotherapy, either based on DLI (recommendation CIII), or infusion of EBV-CTL (recommendation CII) [4]. The latter method results in 88% response, but it requires good laboratory experience and is time-consuming, since 8-10 weeks are necessary to generate EBV-CTL. Also, a potential risk of GVHD induction is faced in the HSCT recipient. New methods of faster and easier EBV-CTL generation are being prepared.

Antiviral therapy, in spite of frequent use, is not recommended in PTLD therapy (recommendation EIII) [9]. Such compounds as acyclovir, ganciclovir, foscarnet and cidofovir are active only in the acute lytic phase of EBV infection, but not in the latent one, presented as PTLD. This is due to lack of thymidine kinase expression, which is the target for antiviral drugs.

Recently published data on a multicentre U.S. study on PTLD therapy in patients after solid organ transplantation show that early use of rituximab significantly improves survival, when compared to other treatment modalities [24]. The risk factors for an unfavourable outcome were central nervous system involvement, bone marrow involvement and hypoalbuminaemia. This report proves that apart from reduction of immunosuppressive therapy, the current practice in first-line therapy of PTLD after SOT is the use of rituximab [24].

Preemptive therapy

Preemptive therapy in patients at risk of EBV-PTLD can be defined as any agents or EBV-specific T-cells given to an asymptomatic patient with EBV-DNAaemia detected by a screening assay. This approach is used in patients with EBV-DNAaemia, but without any lymphoid or extralymphoid mass related to EBV. Preemptive therapy is based on screening of serum EBV-DNAaemia. The aim of preemptive therapy is inhibition of viral replication and prevention of development of symptomatic PTLD [9].

Preemptive therapy is based on rituximab use, which is effective in 89.7% of patients (recommendation AII) [4]. This preemptive strategy is a common practice in 80% of European transplant centres [14]. The use of 1-2 weekly rituximab doses is usually sufficient to stop the viral replication, although the timing of preemptive therapy is a matter of discussion. Usually, it is based on serum viraemia with EBV-DNA copies > 10^3-10^4/ml, or with increasing viral load. A new potential possibility is the gene expression analysis being tested in patients after SOT screened for PTLD [25]. In spite of high efficacy of rituximab in preemptive therapy, significantly exceeding efficacy of treatment of overt proven or probable PTLD, the application of rituximab should be considered carefully due to its impact on the immunological system. As mentioned previously, rituximab intensifies lymphopenia and B-lymphocyte dysfunction, already present after HSCT. This can contribute to development of infectious complications and decrease of treatment efficacy.

Other methods of preemptive therapy include DLI use or EBV-CTL infusion, recommended for second-line therapy. Some patients might benefit from decrease of immunosuppressive therapy.

Prophylaxis

Prophylaxis of EBV-PTLD is based on appropriate donor selection, serologically EBV matched with the graft recipient. The prophylactic use of antivirals is a matter of debate, but probably it is ineffective. Two study groups used rituximab prophylactically in patients at high risk of PTLD after HSCT. Rituximab was administered either at day +3 after alloHSCT (Rovelli *et al.*, EBMT, 2004, abstract P753) or at

day +5 (Bacigallupo *et al.*, ASH, 2008, abstract P2232). However, still in 36% of patients, EBV-DNAaemia was observed during follow-up [4].

Conclusions

Rituximab improved the outcome of patients treated for B-cell lymphomas, as well as PTLD. The drug is effective in preemptive treatment and therapy of documented disease.

Rituximab is a safe drug, but it causes profound B-cell depletion and delay in B-lymphocyte recovery and immune response to EBV.

Preemptive therapy with rituximab is currently the most effective strategy to control EBV-PTLD. Treatment of patients with PTLD who are refractory to rituximab as well as their identification remain a challenge for clinicians. A diagnostic and therapeutic strategy for high risk patients should be implemented in transplant centres performing alloHSCT.

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