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Posttransplant lymphoproliferative disorder in children after allogeneic hematopoietic stem cell transplantation: a single-center experience and literature review

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Summary

Posttransplant lymphoproliferative disorder (PTLD) is one of the most serious complications of allogeneic hematopoietic stem cell transplantation (HSCT). Pathogenesis of this disease is associated with uncontrolled lymphoid tissue proliferation in immunocompromised recipients, most often triggered by primary Epstein-Barr virus infection, or its reactivation. This complication could be fatal, depending on the type of PTLD. This article describes clinical and morphological classification, risk factors, clinical features, diagnostic and treatment of PTLD and presents the clinical experience of the diagnostic and treatment of PTLD in patients of HSCT departments of Russian Children's Hospital and National Scientific Center of Children's Hematology, Oncology and Immunology.

Keywords

Allogeneic hematopoietic stem cell transplantation, posttransplant lymphoproliferative disorder.

Introduction

The number of allogeneic hematopoietic stem cell transplantations (HSCT) continues to increase, including transplants from alternative donors. Therefore, an uncommon HSCT complication called a posttransplant lymphoproliferative disease (PTLD) should be in focus, due to its extreme danger to patients.

Since 60's, lymphoid-derived posttransplant neoplasias were first described in renal transplant patients who received immunosuppressive drugs to prevent graft rejection [45]. PTLD is a common complication in solid organ transplant settings, occuring at a rate of 1 to 20%, being dependent on the graft type [7]. Similarly, PTLD may develop after allo-HSCT presenting many factors predisposing for deficient immune surveillance over proliferating B cells. PLTD incidence following allo-HSCT varies between 0.8 and 1.5% [2]. Some PTLD

cases are described after umbilical blood transplantation [18], and allo-HSCT with nonmyeloablative conditioning [5, 52].

PTLD comprises a group of disorders ranging from benign polyclonal hyperplasia to malignant clonal proliferation [42, 25, 8, 30, 38]. PTLD is historically recognized as uncontrolled B cell proliferation caused by Epstein-Barr virus (EBV). However, EBV-negative PTLD are described as well [29].

Classification

All the posttransplant lymphoid neoplasias were previously called immunoblastic sarcomas until PTLD discretion, as a certain clinical entity. In 1987, Frizzera et al. [17] described some distinct polymorphic changes in patients after renal transplantation, and proposed a classification including a non-specific hyperplasia, polymorphic hyperplasia, and polymorphic lymphoma. In 1988, Nalesnik et al. coined a term *polymorphic PTLD* for the mentioned disorder [39]. Monomorphic PTLD was also described but it could not be differed from a non-Hodgkin's lymphoma. However, mere morphological findings did not provide complete and reliable prognostic information. Knowles et al. [27] added combined molecular genetics criteria to classical morphological features in order to determine cellular clonality, thus developing a PTLD classification including a polyclonal plasmatic hyperplasia, monoclonal polymorphic B cell hyperplasia, or lymphoma, as well as monoclonal pleiomorphic immunoblastic lymphoma, or multiple myeloma.

By 1997, Society for Hematopathology developed a novel classification which initially pointed to differences between early and late PTLD's [24]. In 2001, The World Health Organization (WHO) published current PTLD classification which is used up to present time: 1) initial disturbance, e.g., reactive lymphoplasmacytic hyperplasia, and a syndrome similar to infectious mononucleosis, 2) polymorphic PTLD; 3) monomorphic PTLD, and, 4) Hodgkin's disease-like PTLD (Table 1) [26, 33]. In 2008, this classification was supplemented by additional histological criteria.

Table 1. PTLD categories according to WHO Classification of Tumours [26]

Category	Clonality	Clinical characteristics
Early lesions A) reactive lymphoplasmocytic hyperplasia B) Infectious mononucleosis-like lesions	Polyclonal	Usually spontaneous regression, or following reduced immunosuppression therapy
Polymorphic PTLD	Polyclonal in most cases	Variable response to reduced reduced immunosuppression therapy.
Monomorphic PTLD	Monoclonal	Should be classified according to WHO classification for non-Hodgkin lymphomas
Classical Hodgkin's lymphoma and Hodgkin's lymphoma-like PTLD	Monoclonal	Similar to Hodgkin's lymphoma

Notes: WHO, World Health Organization; PTLD, posttransplant lymphoproliferative disease; NHL, non-Hodgkin's lymphomas.

Etiology and Pathogenesis

Primary EBV infection after transplantation is the main factor of PTLD. I.e., the PTLD risk after EBV infection is shown to be increased 10- to 76-fold [7]. EBV, herpesvirus family member may cause of infectious mononucleosis. Human fluids and secretions, e.g., saliva, are a usual transfection source. Over 90% of humans develop anti-EBV immunity by the age of 40 years. Following primary infection, a long-lasting viral latency is established. An immunocompetent organism has several control mechanisms against EBV proliferation after primary infection, especially, cytotoxic T cell response, and, to lesser degree, humoral (antibody) immune response; NK cell activity, cytokine regulatory pathways [51, 35]. EBV transmission to the HSCT recipients occurs mainly via blood products, however, exact incidence of this transfection is undetermined. In cases of B cell PTLD, B cell proliferation and inhibition of specific immune surveillance are the main causal factors [1]. EBV is known to primarily affect naïve B cells which migrate to germinative centers. Specific EBV proteins are stimulating differentiation of B cells to memory B cells that become the EBV depots. In summary, expression of EBV markers (LMP1, 2A-B), and nuclear proteins (EBNA-1, 2, 3A-C) is accompained by development of the virus latency. These latent gene expression is associated with ongoing EBV infection of B cells, and, accordingly, with different kinds of PTLD [37] (Table 2). Hence, EBV genome in immunocompetent subjects exists as episomes providing latency in mem-

ory B cells. Under inhibited immunity, the T cell control is also lost, thus causing proliferation of EBV-infected B cells, lymphoid cell hyperplasia, and evolving malignancy [32]. T cell recovery does not yet occur within 6 months post-HSCT, thus predisposing for higher PTLD risk during this time period. [4]. However, an increase in late PTLD cases is observed over last years [50]. As a rule, this trend is associated, with low CD4+ lymphocyte levels as it occurs in HIV-infected patients [20].

Table 2. EBV-associated PTLD and viral programs [37]

Latency period	Expressed EBV-specific proteins	B lympocyte development stage	PTLD type
III (growth)	EBER1-2, EBNA1-6, LMP1, LMP2A-B	Activated B-lymphoblasts	Post-transplant diffuse large-cell, B cell lymphoma; HIV-associated lymphoma; Acute infectious mononucleosis
II («silence»)	EBER 1-2, EBNA1, LMP1-2A	B cells in germinative centers	Post-transplant diffuse large-cell B cell lymphoma; Hodgkin's lymphoma
I	EBER 1-2, EBNA1	Memory B cells	Burkitt lymphoma, Post-transplant plasmoblastic lymphoma

In early PTLD (1st year after HSCT) EBV is found in >90% of B cells. With time, a year or later after HSCT, the EBV detectability decreases gradually, reaching an average of 21-32% of total [16]. Over last years, growing number of EBV-negative PTLD's has been registered: from 10% in 90's to 48% over 2008-2013 [34]. Nevertheless, EBV presence is recommended for every bioptate taken using *in situ* hybridization since EBV status determines appropriate therapeutic approaches. Cytomegalovirus and human herpesvirus could be also detected in blood and tissues of the patients, being, however, an epiphenomenon rather than a disease trigger. [6, 62].

When transplanting solid organ, the PTLD emerges from recipient cells. Meanwhile, both donor and recipient in allogeneic HSCT, are EBV-seropositive in most cases. Hence, lymphoproliferation after allo-HSCT originates from donor cells because lymphoid system in recipient is often virtually destroyed by conditioning treatment. Even in cases of EBV-seronegativity in donor, PTLD develop, due to infection of donor lymphocytes from EBV-positive recipient.

Risk factors

In addition to EBV infection, a number of other HSCT-associated risk factors for PTLD are reported, e.g.: HLA-compatible donor (RR 3,8-9); T cell depletion (RR 4-12,7), treatment with CD3 antibodies; usage of antithymocyte globulin (ATG) (RR 3.1-6.4), severe acute GvHD, grade ≥2 (RR 1.9-6.5); extensive chronic GvHD (risk factor for a late PTLD) [2, 53]. As reported by Uhlin et al. [59], incidence of the EBV-associated PTLD may increase to 10-20% upon combination of some known risk factors: HLA mismatch, different EBV serology in donor/recipient pairs; reduced intensity conditioning; acute GvHD; splenectomy before HSCT; mesenchymal stem cell infusions. The EBV viral load in cases of viral reactivation does not play a sufficient role. E.g., PTLD was registered in 50% of the patients with blood EBV contents of ≥4,000 copies per mL [60]. Meanwhile, current European Guidelines recommend weekly quantitative PCR screening for EBV in allo-HSCT recipients for a minimum of 3 months post-HSCT [55].

Despite donor origin of proliferating B cells in most HSCT cases, high prevalence of PTLD is described in pediatric population among patients receiving ATG- or Alemtuzumab-containing conditioning, due to persistence of recipient B cells in this setting [9, 5].

One should not underestimate EBV-negative PTLDs which occur at later terms post-HSCT, showing a more aggressive clinical course [40]. Some authors suggest to consider them as "classic" lymphomas developing in transplanted patients [36]. Interestingly, the results of an international multicentric prospective study (Phase 2) do not consider EBV status a significant factor influencing overall survival and progression terms [57].

Clinical Features

PTLD manifestations may be quite diverse. Lymphadenopathy, or limited affection of lymphoid tissue are most common. Diffuse lesions similar to fulminant septic syndrome may occur more rarely [19]. The disorder may manifest like an acute respiratory viral infection, sometimes exhibiting functional affection of a distinct organ. Many cases could be complicated by cytomegalovirus infection, or by invasive aspergillosis. In some instances, PTLD proceeds symptomless, being detectable as an occasional finding at autopsy. Any HSCT patient presenting with notable adenopathy, bulky lesions, fever, unexplained pain, weight loss, or organ dysfunction should be examined, e.g., for PTLD [32]. Mortality with PTLD reaches 40-70% after solid organ transplantation. Early mortality from PTLD pst HSCT comprised 90% a decade ago. Overall five-year survival has increased to 40-60% by the present time, due to implementation of adoptive cell therapy [11]. Most lethal outcomes are associated with dis-

ease progression. Other 40% of deaths are attributed to infections and therapeutic toxicity. Unfavorable prognosis is associated with older age of the patient, advanced disease stages, bad somatic status, CNS affection, as well as increased LDH levels and hypoalbuminaemia.An International Prognostic Index (IPI) may be used as a predictor in PTLD patients.

Diagnostics

To assess proper diagnosis, EBV detection in blood by means of PCR technique should be used, along with studies of biopsies taken from affected tissues being performed with combined histology, immunophenotyping, immunohistochemistry, molecular techmiques, e.g., *in situ* hybridization of early EBV DNA (EBER), and PCR for EBV. The disorder should be clearly proven, since some treatment modes could cause severe complications in the patients.

In some cases, polymorphic PTLDs is difficult to discern from infectious mononucleosis or Hodgkin's disease which may manifest with similar disorders [12]. Cell infiltrate in pathological samples consists of lymphocytes, histiocytes and plasmocytes. The latters comprise transformed B blasts expressing CD20 and CD30, bieng CD15-negative. Monomorphic PTLD comply with histological criteria of lymphoma, mostly, B phenotype (especially, B cell lymphoma, diffuse large cell lymphoma, plasmoblastic lymphoma). However, T cell variants are also described (e.g., hepatolienal T cell lymphoma), and combined-type lymphomas. Hodgkin's lymphoma after HSCT occurs sporadically, with Hodgkin and Reed-Sternberg cells being an obligate component of cellular substrate containing plasmocytes, eosinophils and histiocytes. The marker cells exhibit high CD30 and CD15 expression with absence of CD20 and weak PAX5 expression [58]. In Hodgkin's-like PTLD, they are more aggressively presented, being in most cases associated with unfavorable prognosis [28, 48, 46]. These four categories are sometimes hardly discernable, due to cross-presentation of different cellular subsets. Lesions at different sites may exhibit distinct pathohistological pattern. Therefore, correlation with clinical and visualization data should be used to make the diagnosis more correct.

Clonality studies help to confirm the diagnosis. I.e., monomorphic PTLD usually exhibits clonal immunoglobulins or TCR rearrangements, respectively, in B and T cell populations. Due to immune suppression, the B cell PTLDs often express oligoclonal reactive T cell populations detectable by PCR for distinct T cell receptors. They could not be considered classical T cell lymphomas despite their lymphoma pattern revealed by histological criteria. For PTLD staging, they use computer tomography (CT) of chest, abdomen and pelvis minor areas, as well serum LDH determination.

To conduct early monitoring of EBV burden before clinical symptoms of the disorder, quantitative PCR of viral DNA from blood serum is performed. However, it does not substitute requirements for local biopsies to perform adequate diagnostics.

Positron emission tomography with fluorodeoxyglucose (F-FDG-PET/CT) is a golden standard, aiming to assess parameters of lesion and its response to therapy. Extreme importance of PET/CT is proven, in order to justify terms of treatment, especially for the patients with incomplete response to therapy [56].

Prophylaxis

The best way to manage PTLD patients is to minimize potential risk factors. E.g., the PTLD risk is sufficiently increased upon usage of anti-CD3 or ATG preparations for T cell depletion, aiming for GvHD control. Respectively, an option of B cell depletion should be considered if such approaches cannot be avoided. Testing anti-EBV antibodies in donors is an obligate requirement. A seropositive donor is a risk factor in case of seronegative recipient. Additional leucocyte reduction of RBC preparations is recommended, thus allowing to decrease risk for EBV-positive blood products [47]. CMV infection is considered to be a cofactor of PTLD development following solid organ transplantation. Therefore, CMV status of donor and recipient is also of great significance.

Rapid T cell reconstitution is a favorable factor. E.g., incidence of EBV viremia, and, accordigly, PTLD risk in ATG-treated HSCT patients proved to be suffificiently lower at T cell levels of >50/mcL by D+30 [44].

Rituximab (an anti-CD20 monoclonal antibody) could be used as prophylaxis [61] and preventive treatment of PTLD. E.g., a weekly qPCR EBV monitoring at the City of Hope Clinics (USA) is performed since D+21 after HSCT [33]. In case if EBV levels exceed 1000 copies/mL, the patient is administered a single Rituximab dose. In case of EBV persistence for 6 other weeks, three Rituximab infusions are preformed in addition.

Acyclovir or Gancyclovir usage was also of some interest. Gancyclovir is active *in vitro* against EBV, however, it may cause a sufficient myelosuppression [31]. The data on its clinical efficiency in PTLD prevention are controversial.

Early studies of EBV-cytotoxic T cell infusions have shown their efficiency for viral load reduction, and those may be used to prevent and treat PTLD [49, 10, 21].

Certainly, B cell depletion of hematopoietic grafts (by means of Rituximab or CD19+ cell depletion) remains the most effective tool for PTLD prevention.

Treatment

Special guidelines for PTLD treatment were designed on the basis of WHO classification [26]. Type 1 PTLD, or early polyclonal disturbances, including reactive lymphoplasmocytic hyperplasia or infectious monucleosis-like syndromes, do not usually require any interventions, being self-limited. However, reduction of immunosuppressive therapy (IST) is recommended in such cases. Type 2 of the polyclonal PTLD usually needs immunosuppression reduction with variable clinical response. Type 3 (lymphoma) is a subject to treatment in case of reduced immunosuppression and chemotherapy applied. Type 4 PTLD requires aggressive therapeutic approach.

Efficiency of reduced immunosuppression in PTLD is described as early as in 1984 [54]. This approach works both in EBV-associated PTLD patients, and in EBV-negative conditions. Absence of clinical response is predicted by LDH increase >2.5-fold over normal values, organ dysfunction, multiple organ failure. However, development or aggravation of acute GvHD could occur due to IST reduction, thus sufficiently worsenig prognosis of the disorder.

Rituximab proved to be an effective preparation in PTLD [3, 41, 15]. It is considered to be a "golden standard" for treatment of CD20+ PTLD including mono- and polymorphic lesions. When transplanting solid organs, full clinical response to Rituximab monotherapy was registered in 53-86% patients [41, 15]. EBV positivity is a predictor of clinical response. The authors recommend reduced immunosuppression and Rituximab admonistration for the patients with EBV-positive PTLD, whereas polychemotherapy (PChT) is reserved for EBV negative, or Rituximab-nonresponding cases. CHOP and ProMACE-CytaBOM are used as chemotherapy regimens for PTLD, like as in non-Hodgkin's lymphoma. This treatment mode remains problematic, due to high risk of severe infections and increased mortality levels.

Despite the Rituximab efficiency, this drug is inefficient in a group of the PTLD patients, whereas PChT application is limited by it's adverse reactions.

Efficiency of cytotoxic EBV-specific T cells was studied in PTLD patients, however, without distinct results [49, 23]. Infusions of native donor lymphocytes may promote restoration of B cell immunity and increase clinical response rates in PTLD to 60-90% [4]. However, only 41% of these patients achieved stable remission. HSCT from EBV-seronegative donors and umbilical blood cells are of limited use in this condition. At the present time, HLA-compatible EBV-specific third-party donor lymphocytes are preferrable, thus suggesting T cell recognition of tumor cells, due to selective restriction of HLA alleles absent from PTLD cells. [13]. However, generation of EBV-specific cytotoxic lymphocytes needs time and expenses, thus limiting clinical usage of this approach. Some workers attempted to develop rapid cultures of EBV-cytotoxic lymphocytes, but their clinical efficiency is not yet proven. At present, donor banks which contain EBV-specific cytotoxic lymphocytes from third-party are arranged. Possible adverse effects may include systemic inflammatory response and minimal GvHD signs. These symptoms fade away upon administration of corticosteroids and Etanecerpt [43]. Cytokine-blocking therapy, e.g., with antibodies against IL-6, a B cell growth stimulant, is described in a Phase I-II multicentric study, showing 41% of clinical response in early PTLD [14, 22]. A concise therapeutic protocol is shown in Fig. 1 [11]. Diverse therapeutic approaches in PTLD are featured in Table 3 [11].

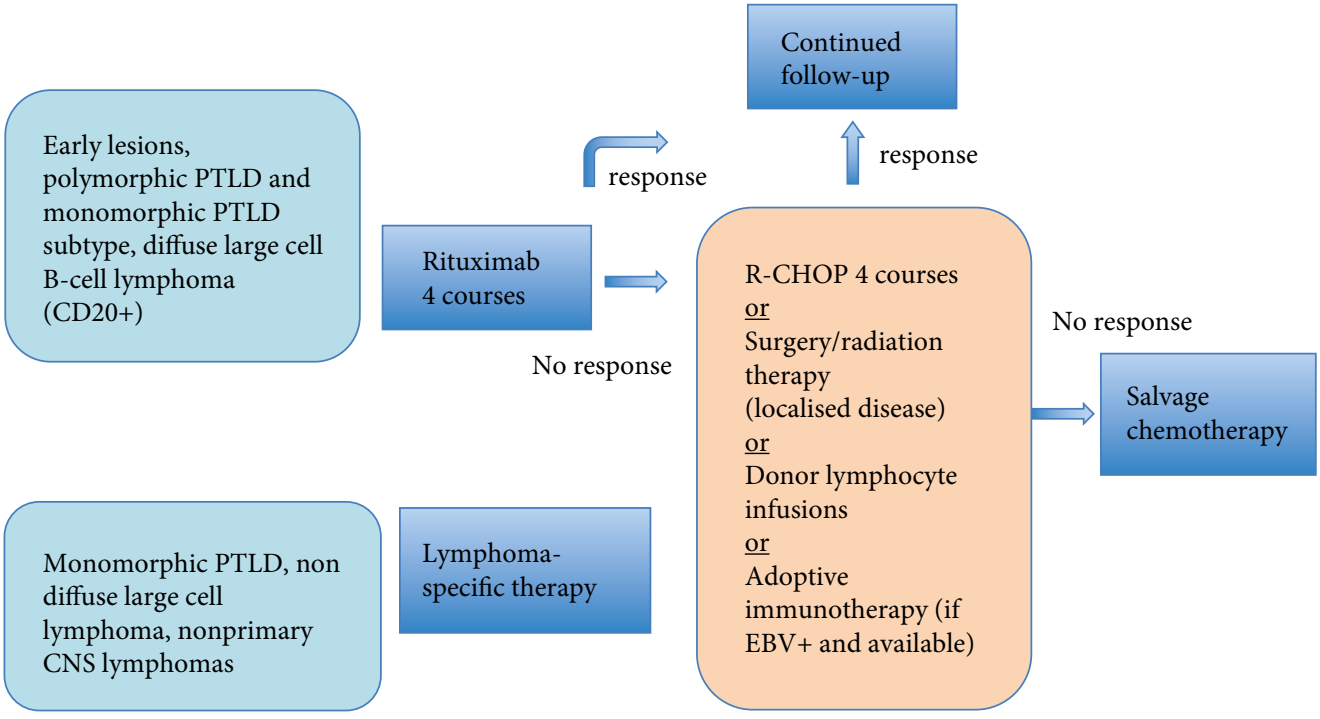


Figure 1. Proposed treatment algorithm for PTLD after HSCT [Dierickx D, Tousseyn T, Gheysens O. How I treat post-transplant lymphoproliferative disorders. Blood. 2015 Nov 12;126(20):2274-83. doi: 10.1182/blood-2015-05-615872. Epub 2015 Sep 17. PMID: 26384356].

Table 3. Treatment options for PTLD [11].

Treatment	Target cell populations	Benefits	Drawbacks
Immunosuppressive therapy reduction	T cells	Good response at early stages of disease. Preventive therapy.	Time-consuming. Depends on organ function. Graft rejection/GvHD risk.
Antibodies against cytokines	T cells Malignant B cells	Promising results for efficient therapy.	High toxicity (side effects).
Donor lymphocyte infusions	T cells (EBV+)	Good response. Rapidly available.	High toxicity (GvHD).
Adoptive immunotherapy (EBV-specific cytotoxic T lymphocytes)	T cells (EBV+)	Promising results in refractory clinical course. Low toxicity. Rapid progress in the field.	Only in EBV+ cases. Time-consuming. High costs. Limited availability.
Surgical treatment and radiation therapy	Malignant B cells	Rapid amelioration of the symptoms.	Only for limited lesions (stage 1). Predominantly palliative treatment.
Cytostatic chemotherapy	Malignant B cells	High response level.	High risk of therapy-associated morbidity and mortality.
Rituximab	Malignant B cells	Good response. Low toxicity. Good performance status retains. Allows to stratify into risk groups. A role in prophylaxis.	Applicable only in CD20+ PTLD. Specific adverse effects (progressing multifocal leukoencephalopathy); hypogammaglobulinemia; Hepatitis B reactivation.
Antiviral drugs	EBV+	Promising role if combined with viral thymidine kinase inducers (arginine butyrate).	Non-effective as monotherapy (thymidine kinase non-expressed in EBV+ PTLD). Only in EBV-positive cases.
Intravenous immunoglobulin (IVIG)	EBV+	Theoretical interest due to precence of EBV-specific antibodies.	As a combination with other treatment methods, absence of data on its real efficiency.

Notes: GvHD, Graft Versus Host Disease; IVIG, intravenous immunoglobulins.

Our clinical experience and discussion

We have analyzed our experience in allogeneic HSCTs performed over 1994-2011 at the Bone Marrow Transplantation Department at the Republican Pediatric Hospital (RPH) and Institute of Children Hematology, as well as allo-HSCTs carried out within 2012-2016 at the Dmitry Rogachev National Scientific and Practical Center of Pediatric Hematology, Oncology and Immunology (Moscow, Russia). From 1994 to 2011, 361 allo-HSCT were performed at the BMT Department, with 27 cases of EBV reactivation (8% of total). Among them, 9 patients showed EBV viremia followed by spontaneous resolution, whereas, in twelve cases, EBV loads required preventive therapy with Rituximab.

In six patients, EBV-associated lymphoproliferative syndrome was observed. Of those PTLD cases, three children received Rituximab treatment with clinical effect; two children required combined therapy with Rituximab and cytostatic chemotherapy. In one child, the disorder proceeded in a fulminant manner, showing no response to Rituximab. Among the group with documented EBV reactivation, eight children have been lost, including three cases of primary disease (1 case was combined with PTLD). In two patients, death was caused by chronic GvHD complicated by infections; in 1 case, lethal outcome was due to heart insufficiency in PTLD with clinical response to Rituximab. One lethal outcome occurred due to multiorgan failure underlied by EBV viremia, and only one case of EBV-associated PTLD proceeded in fulminant manner, with liver and abdominal lymph node affection, thus becoming an immediate cause of death. Clinical characteristics of all patients with EBV reactivation is presented in Table 4. The data on PTLD patients are shown in Table 5.

Table 4. Clinical features of the patients with EBV reactivation

Parameters	Number of patients
Total amount	27
Gender: Boys Girls	 17 10
Age: 0-10 years old over 10 years old	 18 9
Primary diagnosis: Aplastic anemia Acute myeloblastic leukemia Acute biphenotypic leukemia Myelodysplastic syndrome Mucopolysaccharidosis type I Primary immune deficiency Juvenile myelomonocytic leukemia Acute lymphoblastic leukemia Hodgkin's disease Fanconi anemia X-linked adrenoleukodystrophia	 4 4 4 3 3 3 2 1 1 1 1
Donor type: HLA matched related HLA matched unrelated (5, one mismatch; 1, with 2 mismatches) Haploidentical	 8 18 1
Conditioning regimens: Busulfan-containing, Of them with Melphalan Treosulfan-containing Nonmyeloablative, Fludara-containing	 17 8 5 5
GvHD prophylaxis: Cyclosporine+Mycophenolate mofetil Prograph+Mycophenolate mofetil Cyclosporine+ Methotrexate Corticosteroids	 10 15 1 1
Engraftment	25
Acute GvHD, stage 2-4	16
Chronic GvHD: Total case number Limited Extensive	 16 4 12
CMV reactivation	13
EBV viremia, non-treated	9
EBV viremia, Rituximab preventive therapy	12
EBV-PTLD: Total case number Rituximab treatment Rituximab + chemotherapy treatment	 6 4 2
Mortality: Total case number Relapse Chronic GvHD + infections Heart insufficiency (EBV-PTLD in anamnesis) Multiorgan failure EBV-PTLD	 8 3 2 1 1 1

Table 5. Characteristics of the EBV-PTLD patients

Characteristics	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age at the time of HSCT, years	3	1,5	12	11	10	4
Gender	female	male	male	male	male	male
Diagnosis	ALL	AML	AA	MDS, RAEB	AA	Acute biphenotypic leukemia
Donor type	MUD	MUD	MUD (B-mismatch)	MUD	MRD (repeated HSCT)	MUD
EBV status, (serology in patient/donor)	+/+	+/+	+/+	+/+	+/+	+/-
Conditioning regimen	Treosulfan, Fludarabine-Tiothepa, ATG	Treosulfan, Fludarabine, Melphalan, Thymoglobulin	TAO, Cyclophosphamide, Fludarabine, Thymoglobulin	Busulfan, Fludarabine, Thymoglobulin	TAO, Cyclophosphamide, Fludarabine, Thymoglobulin	Busulfan, Fludarabine, Melphalan, Thymoglobulin
GvHD prophylaxis	Cyclosporin + Methotrexate	Tacrolimus, Mycophenolate mofetil	Tacrolimus, Mycophenolate mofetil	Tacrolimus, Mycophenolate mofetil	Tacrolimus, Mycophenolate mofetil	Tacrolimus, Mycophenolate mofetil
Graft cellularity, nucleated cells (x10^8/kg)	6,5	9	5	6	4,6	7
Engraftment, days	12	33	22	No	27	16
Acute GvHD	Grade 1	0	Grade 1	0	0	Grade 1
Chronic GvHD	0	0	Skin and liver lesions	0	0	0
CMV reactivation	No	Yes, CMV pneumonia	Yes	Yes	No	Yes
Graft problems	No	Relapse at 5 months	No	Graft failure, autoimmune hemolysis, Hypofunction of 2nd graft	Rejection	No
Repeated HSCT				Repeated HSCT with Alemtuzumab	Repeated HSCT	
Long-term immunosuppressive therapy (corticosteroiods >6 months)	No	No	Yes	Yes	No	No
Terms of PTLD development, months	4 mo	3 mo	4 mo	4 months after repeated HSCT	1 month after repeated HSCT	2 mo
Clinical presentation	EBV viremia (high), acute hepatitis, enlarged abdominal lymph nodes	EBV viremia, lymphadenopathy, lymph node conglomerate in abdominal cavity, EBV encephalitis	EBV viremia, fever, lymph node conglomerate in left cervical area, soft mass in oropharyngeal area	Lymphadenopathy, hepatosplenomegaly	Lymphoproliferation (oropharynx, cervical lymph nodes)	EBV viremia, EBV-associated encephalitis

Characteristics	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Diagnostics	EBV (PCR)	EBV (PCR) (blood)	EBV (PCR) (blood) Lymph node bioptate histology, EBV (PCR) in biopsy, immunohistochemical (B cell markers of PTLD)	Rising IgG, oligoclonality	EBV (PCR) (blood), Lymph node bioptate histology: plasma cell hyperplasia, EBV (PCR) in bioptate	EBV (PCR) (blood, cerebrospinal fluid)
First-line treatment	Rituximab №2	Rituximab №4	Immunosupression therapy discontinued Rituximab №4	Rituximab №4	Immunosupression therapy discontinued Rituximab №4	Rituximab №4
Second-line treatment	No	Donor lymphocyte infusions №3 IVIG	CHOP №2 Donor lymphocyte infusion №2 EBV-specific donor cells №5	No	No	Rituximab intralumbar, High-dose Methotrexate
Response to therapy	No	No	Response to adoptive cell therapy	To the 1st line treatment	To the 1st line treatment	To the 2nd line treatment
Outcome	Death 3 weeks from manifesting EBV viremia	Relapse, death 6 months from HSCT	Alive	Death with heart insufficiency	Alive	Alive, hemiparesis, symptomatic epilepsy

Below, we would like to report a detailed description of the most severe clinical case where all available therapeutic options were applied (Patient 3).

Clinical case description

A boy with immune thrombocytopenia diagnosed at 6 years, received corticosteroids without effect; intravenous immunoglobulins (IVIG) with minimal effect. At the age of 10 years, the disorder was complicated by anemia and leukopenia. At the RPH Department of General Hematology, the diagnosis was formulated as follows: acquired idiopathic aplastic anemia, a supersevere form. Due to absence of related compatible donor, immunosupressive therapy was performed with cyclosporine, ATG (2 rounds), without any clinical effect. Multiple transfusions were complicated by hemosiderosis.

At the age of 12 years, the child underwent allogeneic hematopoietic stem cell transplantation from a compatible unrelated donor (9/10 antigens, mismatch for a B locus) with minor ABO incombatibility, and EBV VCA IgG positivity in both donor and recipient. Conditioning regimen consisted of thoraco-abdominal irradiation at a dose of 2 Gy; Fludarabine, 150 mg/m^2, Cyclophosphamide, 100 mg/kg; Thymoglobulin, 10 mg/kg (total doses are shown). Graft characteristics: nucleated cells, 5x10^8/kg; CD34+ cells, 3.14x10^6/kg. GvHD prophylaxis was performed with Tacrolimus and Mycophenolate mofetil.

Engraftment was registered at the day +22. Early posttransplant period was complicated by febrile neutropenia. Donor chimerism was developed at 2 months; blood group was changed to donor RBCs. Stage 1/2 acute GvHD was registered as skin affection, thus requiring Prednisolone administration for 1 month. In parallel, cytomegalovirus in blood was detectable, having been treated by Gancyclovir. Three months after HSCT, the patient developed persistent fever without response to antibiotics, as well as enlargement of left cervical lymph nodes. EBV viremia (2000 copies/mL) was first registered 2 weeks after these manifestations. Enhanced antibiotic therapy was without effect, the patient's condition became worse, febrile state persisted, accompanied by weakness, asthenia, cachexia. Lymph nodes at the neck area were enlarged, forming a solid conglomerate up to 5 cm in diameter. Lymph node biopsies were performed, followed by their examination at different reference centers (RPH, Moscow; Bureau for Pathology&Anatomy, St. Petersburg). EBV was detected there by means of PCR. Histological pattern corresponded to monomorphic (Moscow), or polymorphic PTLD (St. Petersburg).

In Fig. 2, the results obtained at the Pathology Laboratory in St. Petersburg (Chief, Dr. Yu. A. Krivolapov). A lymphoid tissue fragment exhibited a pattern of lost organ structure. The tissue consisted of diffuse lymphoid cell fields with detectable small and medium-sized lymphocytes, plasmoblasts

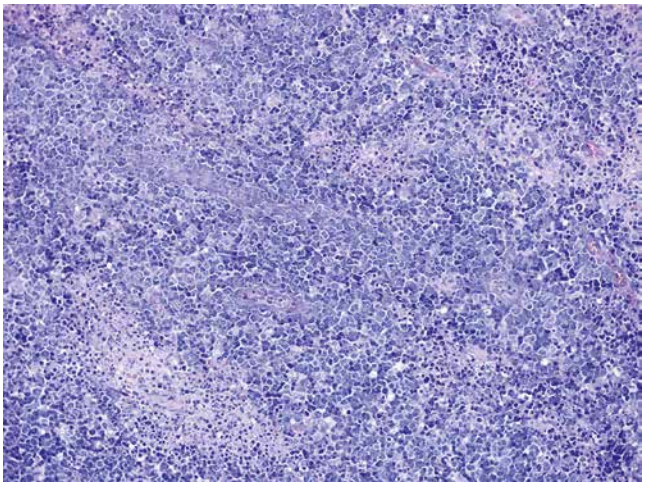


Figure 2. Lymphoid tissue fragment with the loss of topographical structure and polymorphic cell infiltration; hematoxylin and eosin stain, ×40

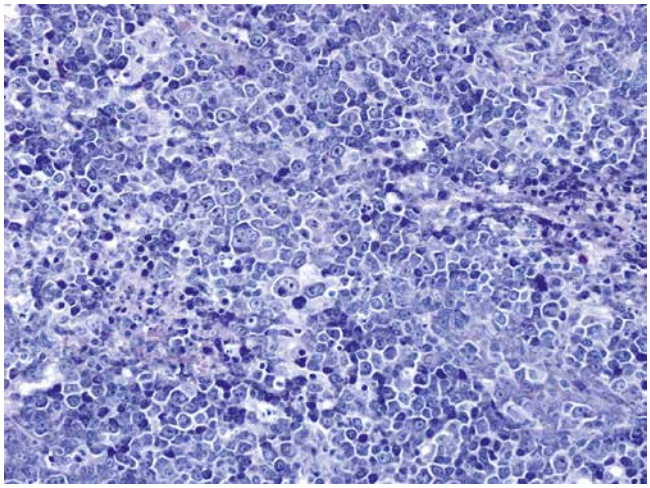


Figure 3. Polymorphic cell infiltrate; hematoxylin and eosin stain, ×40

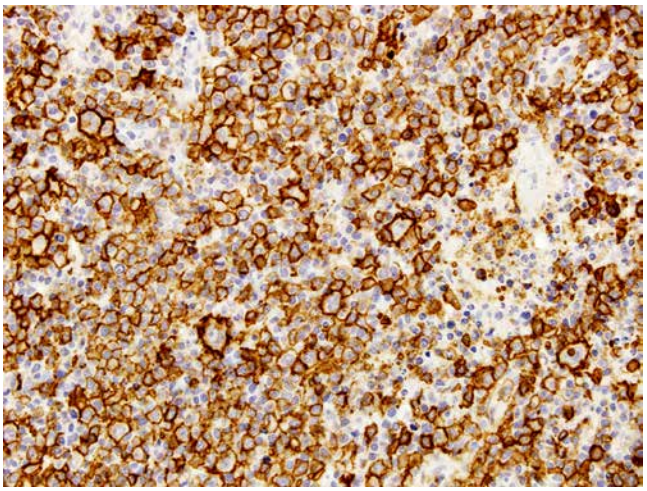


Figure 4. CD20 expression on the tumor cells. Immunohistochemical reaction; ×40

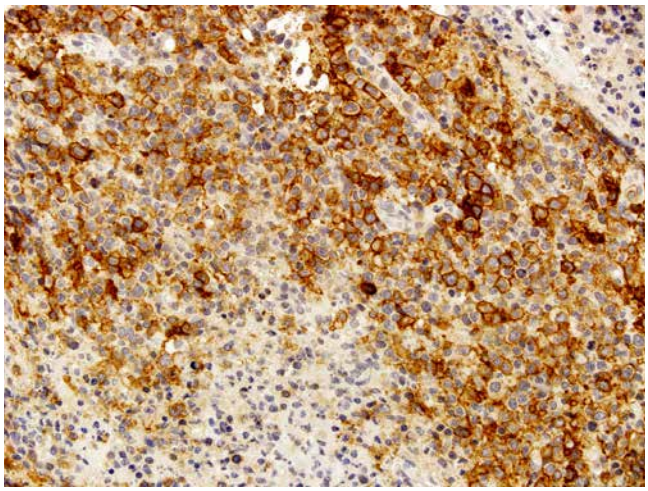


Figure 5. CD30 expression on the tumor cells. Immunohistochemical reaction; ×40

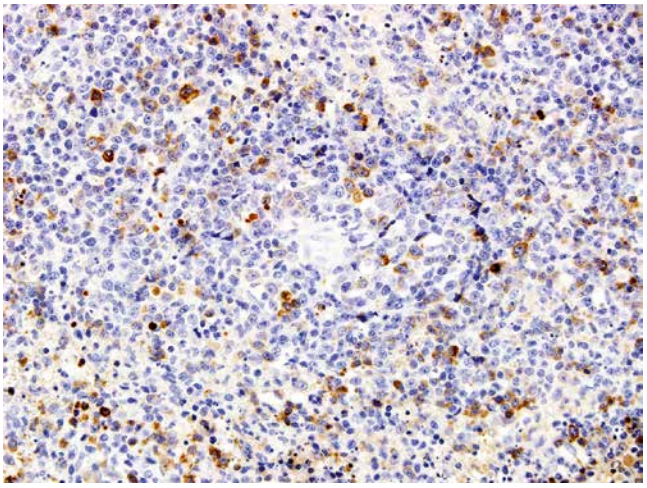


Figure 6. Kappa expression on the tumor cells. Immunohistochemical reaction; ×40

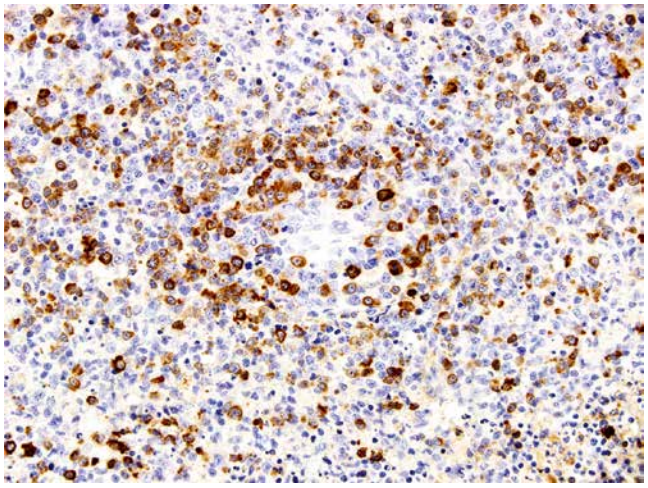


Figure 7. Lambda expression on the tumor cells. Immunohistochemical reaction; ×40

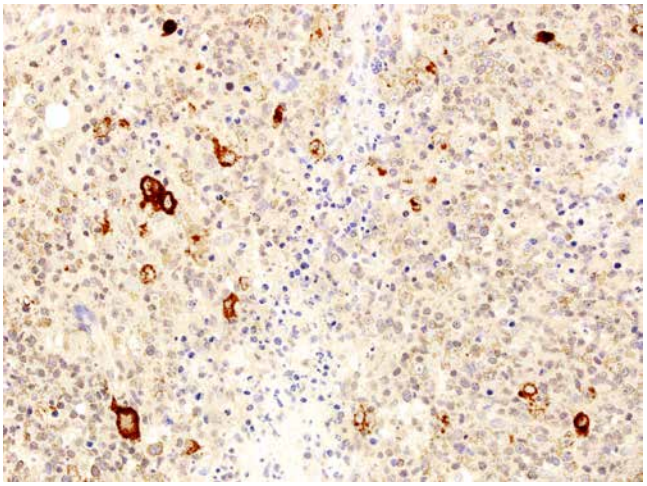


Figure 8. LMP1 expression on the tumor cells. Immunohistochemical reaction; ×40

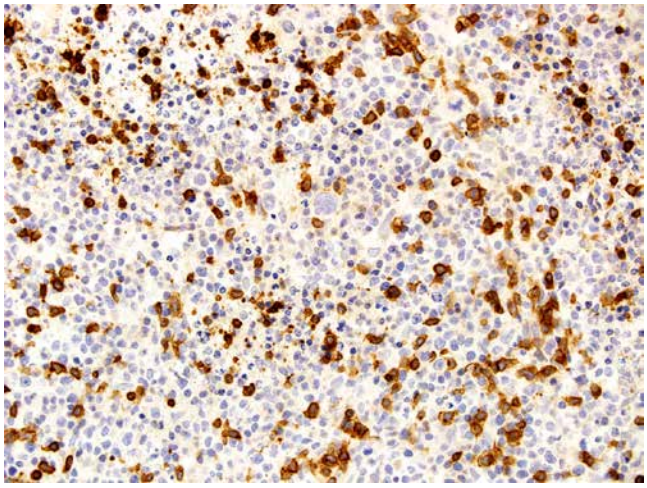


Figure 9. CD3 expression on the tumor cells. Immunohistochemical reaction; ×40

and immunoblasts, large atypical cells with giant, sometimes deformed nuclei with large homogenous nucleoli. Nearly all cells in the field have intensively basophilic cytoplasm (Fig. 3). Mitotic figures are observed. Numerous necrotic foci are revealed, with nuclear fragments (karyorrhexis). Upon immunohistochemical study, vast majority of proliferating cells expressed CD79a (JCB117) and MuM1(Mum1p), with lesser amounts of CD20 (L26)- positive lymphoid cells (Fig. 4). Activated lymphoid cells expressed CD30 (Ber-H2) (Fig. 5). Immunoglobulin light lambda chain-expressing lymphoid cells prevailed over kappa-positive cells in the samples of proliferating tissues (Fig. 6, 7). Large deformed immunoblasts are found there, being both kappa- and lambda-positive. Their cytoplasm showed intensive expression of latent EBV membrane LMP-1(CS1-4) protein (Fig. 8). A proliferative Ki-S5 antigen was expressed in nuclei of ca. 70% of lymphoid cells. Few CD3+ T cells were seen (Fig. 9), with CD8(1A5) cells being prevalent over CD4(4B12)+ lymphocytes. The proliferating tissue did not contain detectable lymphoid cells expressing CALLA CD10 (56C6), or (ALK-1). Ziel-Nilsen Acid fast stain of slices with carbol fuchsin did not show acid-resistant bacteria. Staining with antibodies for M.bovis did not show this antigen. Clinical pattern of the disease, histological structure of lymphoid tissue under study, and immune histochemistry results correspond to polymorphic post-transplant lymphoproliferative disease.

Immunosuppression was discontinued as a first-line therapeutic measure, and treatment with Rituximab was started. Following 4 injections, clinical effect was not reached. Therefore, we undertook a second-line therapy which consisted of a single-block CHOP chemotherapy, which was complicated by enteroparesis. Within first days of chemotherapy, a decrease and softening of the lymph node conglomerate was registered, then followed by the tumor stabilization, with persisting febrile state.

We then started block A (Dexamethasone+Ifosfamide+ Methotrexate, 1 g/m^2 over 24 h + Cytosar + Vepeside, without Vincristine, due to recently observed neuropathy), accompanied by combined anti-infectious therapy. Despite treatment, the neck conglomerate was enlarged, along with continuous febrility. However, EBV was not more detectable in blood by means of PCR. Hence, this case of EBV-associated PTLD was considered refractory. A third block of polychemotherapy was scheduled, as follows: Gemsar, 1 g/m^2 (days 1-6); Carboplatine, 200 mg/m^2 (days 2-5); Vepesid, 150 mg/m^2 (days 2-5); Dexamethasone, 6 mg/m^2 (days 1-6), followed by subsequent transfusion of donor hematopoietic cells (boost without conditioning): on day 3 after finishing therapy, the patient received CD34+ cells at a dose of 11x10^6/kg, and CD3+ cells at a dose of 1x10^4/kg. Two weeks later, the fever faded away, and hematopoiesis recovered. However, the boy showed signs of GvHD: dry skin, exfoliation, hyperpigmentation, weak itching. Nevertheless, a decision was taken to continue donor lymphocyte infusions (DLI). Three weeks after first lymphocyte infusion, a second DLI was performed (CD3+ cells, 5x10^4/kg). Febrile state did resume, but the neck lymph node conglomerate was reduced in size, and hepatosplenomegaly retained. Liver enzyme markers became increased to 400 U/L (ALT and AST); alcaline phosphatase, to 1400 U/L). Toxic hepatitis was diagnosed, and hepatotoxic drugs were withdrawn. However, the condition of patient became worse, i.e., loss of appetite and weight, enteric symptomes occurred, along with icterus and hepatosplenomegaly (liver +8 cm, spleen +2 cm). Blood biochemistry: total bilirubin of 84 mcmol/L; ALT, 1060 U/L, AST, 2217 U/L, alcaline phosphatase, 2630 U/L. Spot/papule eruptions developed at the the skin of head, trunk, as well as mucosal leukoplakia, and intestinal syndrome considered as grade 3 GvHD, with skin, mucosae, liver, intestinal tract lesions consequent to DLI. Corticosteroid treatment was resumed, at 2 mg/kg/day. As result, eruptions were entirely reduced, like as fever, vomitimg and nausea. However, fatigue, low appetite, intestinal syndrome, signs of sinusitis, lung and intestinal infections (cytomegaloviral and adenoviral colitis). The patient received massive combined antibacterial and antiviral therapy (Cydofovir), antifungal treatment.

PTLD features were still detectable in MRI: heterogenous, thickened, soft, contrast-accumulating tissue retained in nasopharinx area, posterior nasal passages; posterior oropharynx (more at right side) looks deformed, mandibular lymph nodes were enlarged on the right. A heterogenous soft tis-

sue mass persisted in lateral part of neck (left side, 18x9x31 mm in size), containing highly dense inclusions (microcalcinates), without proven contrast accumulation. Later on, a volumic decrease in lymphoproliferative changes was noted.

One month later, glucocorticoids were gradually tapered and fully discontinued. Rapamycin was administered as a basic immunosuppressive drug, aiming for immunotherapy, along with gamma-Interferon (2 injections). Clinical condition of the patient remained quite severe being characterized by cachexia, fever, adynamia, graft hypofunction with transfusion demands and requirements for hematopoiesis stimulation. Remarkable cholestasis was also documented (total bilirubin, 256 mcmol/L (direct,162); ALT, 147 U/L; AST, 174 U/L; alcaline phosphatase, 1224 U/L; GGTP, 1372 U/L), like as hemosiderosis (ferritin, 46545 mcg/L).

From these data, we suggested a secondary hemophagocytic syndrome underlied by EBV infection in immunocompromised patient subjected to unrelated allo-HSCT. Dexamethasone therapy was started (10 mg/m^2 №12), Vepesid (150 mg/m^2 twice a week). Fever was stopped, and the size of liver and spleen was diminished. However, infectiuos complications still progressed, along with hypoalbuminemia and oedemas. Antibacterial and accessory treatment was further modified. E.g., grafting of CD34+ cells (10x10^6/kg) was performed, aiming for acceleration of hematopoiesis recovery. During the therapy, small positive changes were documented as decrease of febrile rises, reduced abdominal pains. IST was continued with Rapamycin, and substitutive IVIG transfusions at higher doses were performed, biphosphonates were also administered.

MRI of laryngo-pharyngeal area 8 months after starting PTLD therapy, showed that the right oropharinx, left nasal passages, and left cervical area retain soft tissue lesions; some features of lymphoproliferative lesions in maxillar sinus are also present. By the present, EBV viremia comprised 600 copies/mL, followed by increase to 4320 copies/mL. In parallel, CMV-viremia did also elevate. Therapy with EBV-specific lymphocytes from the same donor was scheduled.

During the waiting period, due to problems with breathing and swallowing, the mass in oropharynx was removed preceded by tracheostoma mounting. Clinical state remained very severe due to infectious complications underlied by pancytopenia and cholestasis syndromes. A month later, the tracheostome was removed. Therapy with EBV-specific donor cytotoxic lymphocytes was commenced (a total of five injections weekly). The therapy was associated with diminished lymph nodes, gradual improvement of blood counts, as well as slow decrease in liver toxicity markers, EBV viremia. Immune reconstitution seemed to proceed with time.

1.5 years after HSCT, there were no additional data for active PTLD (i.e., a year and 3 months after beginning the therapy), main problems concerned hepatic dysfunction and hepatosplenomegaly, along with liver fidrosis and hemochromatosis. The patient has received a long-term therapy with Budenofalk and Exjade.

Subsequently, gradual recovery of somatic status was observed, the boy underwent regular control examinations, replacement therapy with IVIG. His state stabilized 2 years after HSCT. There retaine hepatosplenomegaly, slight increase in hepatic transaminases and alcaline phosphatase. Budenofalk was continued for 3 years. Age-dependent vaccination was performed. At the present time, 10 years after allogeneic HSCT, clinical state of the adolescent is satisfactory, he is learning and keeps active life.

The above clinical description demonstrates an extremely aggressive course of some PTLD cases, thus requiring rapid and precise actions from the doctors. The pathological process developed within typical terms (3 months after HSCT), in absence of immune reconstitution, and exhibited and manifested as an infectious condition with fever and lymphadenopathy. Despite limited localization (oropharynx and cervical regions), the disorder proved to be refractory and threatened with asphyxia at certain stage of disease. Appropriate diagnostics required combined diagnostic measures with dominating histochemical results. Despite a divergent interpretation of mono- or polymorphic lesions in the given EBV-associated PTLD, clinical course and somatic status of the patient determined a vitla demand for changes and careful selection of adequate therapy. One should note professionalism of the medical team, as well as precise actions, patience and insistence of the doctor that determined favorable outcome of this case which initially presented a life-threatening situation.

Meanwhile, the first case presented in Table 5 concerns fulminant course of EBV-PTLD. A 3-year old girl with acute lymphoblastic leukemia (ALL) was subject to allo-HSCT from HLA-compatible, EBV-positive unrelated donor with partial CD34+ graft enrichment. EBV viremia in the patient was registered at 4 months posttransplant, reaching 12,000 copies/mL. A week later, the viremia was increased to 500,000 copies/mL, accompanied by fever; liver damage as documented by growth in transaminases, rising bilirubin; enlarged abdominal lymph nodes. After two Rituximab injections, no positive effect was reached, her condition deteriorated rapidly, and the patient died due to progressing hepatic and respiratory failure. Only three weeks passed since EBV viremia was registered in the girl. Two-week therapy with Rituximab proved to be without any effect. This type of EBV-PTLD (any histology data are not available, due to lacking autopsy) showed a quite aggressive and rapid course, thus preventing alternative therapeutic options. Other PTLD cases observed (see Table 5) depict more favorable variants of the disorder, with positive response to the IST reduction and Rituximab treatment. Interestingly, the patient No.6 developed EBV-associated PTLD despite EBV-seronegativity in his donor, may be, due to endogenous infection of donor cells from recipient. Another unusual presentation of PTLD was connected with affection of central nervous system. However, there was no opportunity to perform stereotactic brain biopsy at the time of encephalitis manifestation. Later on, a biopsy did not reveal an initial cellular substrate. However, a marked response to Rituximab, e.g., its endolumbar injections, and to Methotrexate therapy are indicative for malignant origin of primary CNS lesions in the given patient.

A study of the second cohort of patients who received allo-HSCT from 2012 to 2016 at the NCPHOI has revealed only two EBV-PTLD cases among 911 children (Table 6). This cohort was analysed separately, because the transplants were performed mostly by a novel protocol with a CD19 depletion and inclusion of Rituximab into the conditioning regimen. Among 483 patients after HSCT with alpha/beta depletion and CD19-negative selection, as well as among 316 children who received Rituximab, no single case of PTLD was registered. However, B cell depletion was not performed in the two belowmentioned cases: the first patient was grafted with umbilical blood from unrelated donor. The second patient received bone marrow from a sibling. Therefore, their conditioning regimens were classic, with Thymoglobulin application which is considered an accessory risk factor for PTLD. Description, of these 2 cases, PTLD manifestations and their treatment are seen from Table 6.

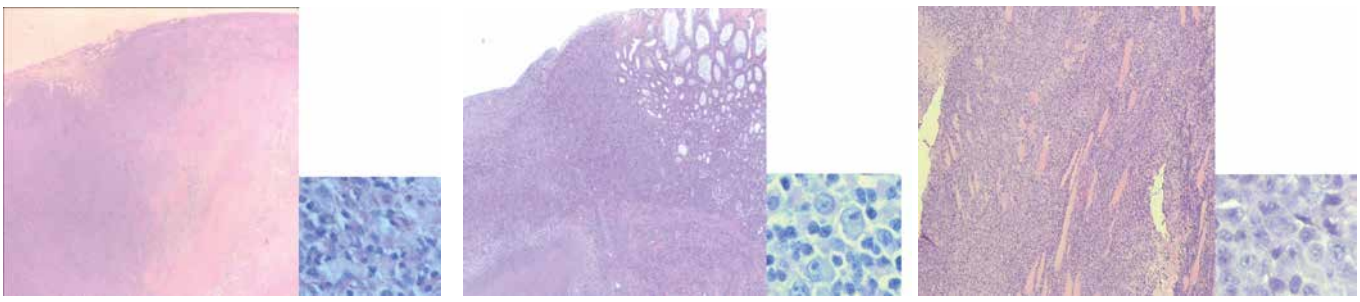
Table 6. Characteristics of the two patients with monomorphic B cell PTLD

Characteristics	Patient 1	Patient 2
Age at the time of HSCT, years	8	14
Gender	Girl	Boy
Diagnosis	Acute myeloblastic leukemia	Aplastic anemia
Donor type	MUD (umbilical blood compatible for 6/10 antigens)	MRD
EBV state (serology) in patient/donor pairs	+/-	+/+
Conditioning regimen	Treosulfan, Fludarabine, Melphalan, Thymoglobulin	Fludarabine, Cyclophosphamide, Thymoglobulin
GvHD prophylaxis	Tacrolimus+corticosteroids	Cyclosporin+ Mycophenolate mofetil
Graft cellularity, nucleated cells (x10^8/kg)	6.5	3.54
Engraftment, days	12	12
Acute GvHD	Grade 2, skin and intestinal affection	0
Chronic GvHD	Intestinal immune lesion, abdominal pains	0
CMV reactivation	Yes	No
Graft problems	Graft failure	No
Repeated HSCT	Mesenchymal stem cells from 3rd party №5	No
Prolonged immunosuppression (corticosteroids > 6 months)	Yes	No
Terms of PTLD development	5 months	Begun since 2 months, progression with culmination by 4 months.
Clinical presentation	Lesions of tonsils, cervical lymph nodes, liver, spleen, intestines, lungs. Febrile state.	EBV viremia 2 months later, foci in liver and spleen, enlarged intra-abdominal lymph nodes. Ulcer of esophageal/cardiac junction. 4 months later, enlargement of submandibular lymph nodes, left tonsil, bulky mass at the left parapharyngeal area. Generalization of the process, relapsing course.
Diagnostics	EBV (PCR) in tonsillar tissue; Histology of a removed tonsil: monomorphic B cell PTLD (DLCBCL NOS tygpe)	EBV (PCR) in tonsillar tissue. Histology (biopsy from submandibular lymph node): EBV-associated PTLD Biopsy of parapharyngeal mass: EBV+ diffuse large-cell B cell lymphoma (monomorphic PTLD). MRI, PET.

Characteristics	Patient 1	Patient 2
First-line treatment	Rituximab №4	Rituximab №4: preventive therapy, refractoryness Cytosar, 6 g/m^2 №3 Etoposide, 125 mg/m^2 №4 Intratecal injection of Cytosar, Methotrexate, Dexamethasone, Anti-CD20 (Gasyva) №1
Second-line treatment	Actemra (anti-IL6 antibody) IVIG Rituximab №4 (2nd course)	Dexamethasone Chemotherapy (CHOP) Brentuximab Vedotin №1 Nivolumab (anti-PD1) №5 Actemra №1 Radiation therapy, at a total dose of 46 Gy DLI №1 (CD45RA-)
Response	Partial	Yes
Outcome	Persistence of a compound disorder (chronic GvHD, EBV-PTLD, poor performance state).	Condition became stable over 1 year and 2 months after HSCT, 8 months of the EBV-PTLD therapy.

These two cases also refer to aggressive and malignant clinical forms of PTLD. The first case concerned a girl with acute myeloblastic leukemia following allografting and development of refractory acute and chronic GvHD without any options of immunotherapy. The B cell monomorphic PTLD did partially respond to Rituximab treatment. More active treatment modes were impossible, due to poor somatic condition of thefemale patient.

In the boy with aplastic anemia, we have documented all stages of EBV-PTLD emergence, including progression from EBV viremia and lymphadenopathy to mucosal lesions (bleeding gastric ulceration requiring partial stomach resection, tonsillar involvement) followed by outgrowth of parapharyngeal tumor mass. We were also able to confirm histologically a transition from polymorphic PTLD to monomorphic aggressive form being similar to malignant large-cell lymphoma by B cell origin (Fig. 10). Such clinical course is rarely described in details, both for clinical and histological pattern, hence this case seems to be original, due to concordance between evolution of modifying pathological pattern and specific treatment mode. At the stage of EBV-associated lymphoadenopathy, a standard approach with Rituximab therapy was applied, however, without effect. This monomorphic PTLD was refractory to therapy with anti-CD20 antibodies. At the next stage, the EBV-PTLD proceeded as a malignant B cell large-cell lymphoma (Fig. 11), this requiring a high-dose chemotherapy. In future, standard polychemotherapy proved to be insuffisient, and clinical effect was obtained only from combined chemotherapy, immune drugs and donor lymphocyte infusion. Nivolumab and Brentuximab were used as a pioneering approach to treatment of such condition. In both children, antibodies against IL-6 were also used with proven effect, in order to ameliorate clinical symptoms.



a) b) c)

Figure 10. Pathomorphosis of PTLD in one patient.

a. hematoxylin and eosin stain; x10, x40. Early PTLD lymph node lesion. The loss of topographic structure, focuses of necrosis, polymorphic cell infiltrate with large EBV-positive cells.

b. hematoxylin and eosin stain; x10, x40. Polymorhic PTLD, mucocutaneous ulcer of the antral stomach. The mucose of the antral stomach with ulceration and a massive transmural infiltration of lamina propria. Polymorphic cell infiltrate with numerous EBV-positive large cells, plasmacytic cells and plasmoblasts, small CD3/CD8 reactive T-lymphocytes.

c. hematoxylin and eosin stain; x20, x40. Monomorphic B-cell PTLD, diffuse large cell B-cell lymphoma. Monomorphic large cell infiltrate with the diffuse distribution among the muscled fibers. Cells with a high mytotic activity – immunoblasts and centroblasts.

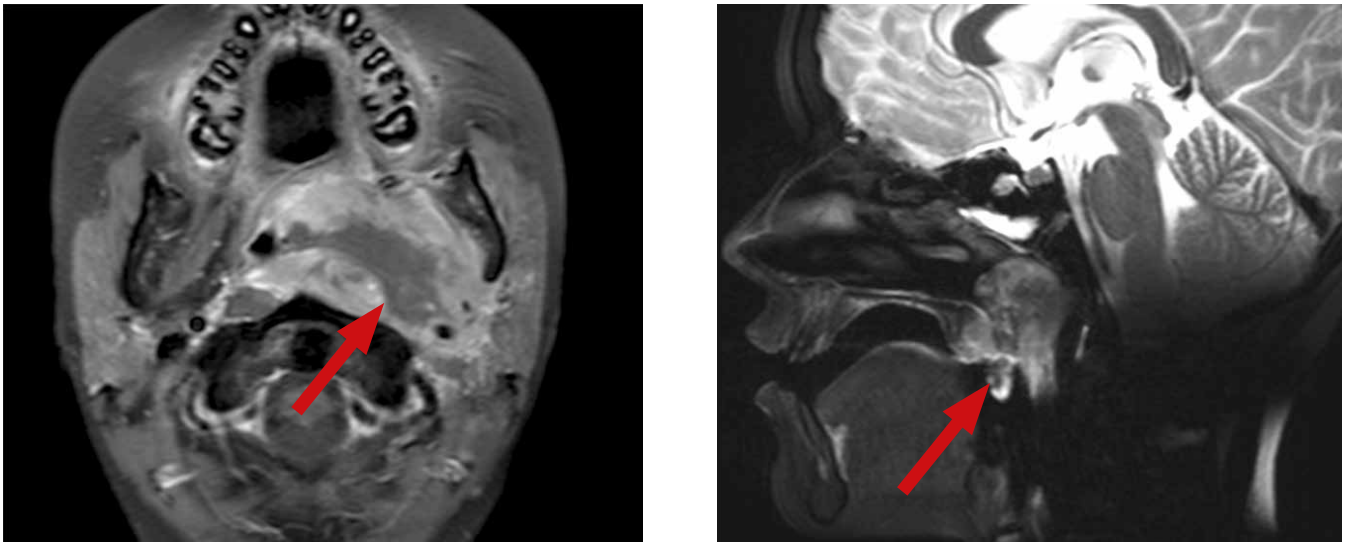


Figure 11. MRI in T1-VI with contrast enchancement and fat supression in axial projection and T2-VI with fat supression in sagittal projection. Tumor mass of the lateropharyngeal space on the left with ingomogenius intensive contrast accumulation; left tonsilla invlovement, nasal pharynx and pharynx invasion.

Conclusion

Hence, PTLD is a challenging pathological process which lets a lot of open questions be answered by appropriate specialists. This complication still bears a risk of high mortality, thus requiring further activities for studying pathogenesis and treatment modes for PTLD. Multicenter research and clinical studies are necessary to evaluate this clinical entity. The PTLD therapy represents an excellent clinical model for combined application of immune therapy, cellular therapy, and standard cytostatic treatment of malignancies which may be used for treatment of other neoplasias and severe viral infections.

Conflict of interest

No conflict of interests is declared.

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Посттрансплантационное лимфопролиферативное заболевание у детей после аллогенной трансплантации гемопоэтических стволовых клеток: опыт центра и обзор литературы

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Резюме

Среди различных осложнений аллогенной трансплантации гемопоэтических стволовых клеток (ТГСК) одним из самых тяжелых является посттрансплантационное лимфопролиферативное заболевание (ПТЛПЗ), проявляющееся неконтролируемой пролиферацией лимфоидной ткани. Пусковым механизмом, как правило, служит первичная инфекция, вызванная Эпштейн-Барр вирусом, или реактивация вируса в иммунокомпрометированном организме. В зависимости от вида ПТЛПЗ и динамики его развития данная патология может быть фатальной для пациента. В данной статье описаны: клинико-морфологическая классификация, факторы риска, клинические особенности, диагностика и лечение ПТЛПЗ, а также приведен клинический опыт диагностики и лечения данного осложнения на базе отделений ТГСК РДКБ и ННПЦ ДГОИ.

Ключевые слова

Аллогенная трансплантация гемопоэтических стволовых клеток, посттрансплантационное лимфопролиферативное заболевание.