Lymphoproliferative disorder in a twin female teenager post kidney transplantation

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Abstract
Post-transplant lymphoproliferative disorder (PTLD) is defined as a heterogeneous group of lymphoid and plasmocytic proliferations with variable malignant potential. They often arise in immunocompromised post solid organ transplant (SOT) patients linked with Epstein–Barr virus (EBV) infection. Clinical manifestations include fever, lymphadenopathy and organ involvement. Diagnosis of PTLD requires morpho-pathological tissue examination. Treatment of EBV-related PTLD in SOT patients includes immunosuppressive (IS) agents' reduction, use of antiviral medication, anti-B-lymphocyte antibodies and chemotherapy for high-risk patients. We report a case of late EBV-related PTLD occurring in a young female, coming from twins, nine years after renal transplant from deceased donor. Both sisters were diagnosed at the age of 10 with chronic kidney disease (CKD) based on nephronophthisis and underwent the first simultaneous renal transplant from deceased donor in Romania. PTLD Hodgkin’s-like lymphoma and EBV-positive lesions were to be found in autopsy. Routine EBV viral load testing and immune condition in SOT patients could identify PTLD risk factors therefore early treatment can be applied. Monitoring EBV serology and immunological parameters are preferred as strategy for PTLD prevention.

Keywords: kidney transplantation, twins, Epstein–Barr virus, lymphoma, nephronophthisis.

Introduction
Latest improvements in renal transplantation and other solid organ transplantation (SOT) brought a better prognosis in pediatric population with hereditary or acquired chronic kidney disease (CKD). Immunosuppressive (IS) agents used to prevent graft rejection induce a greater risk of infections and also cancer development [1]. One of the most frequent malignant complications in pediatric population after SOT is a group of diseases under the name of post-transplant lymphoproliferative disorder (PTLD). They often arise associated with Epstein–Barr virus (EBV) infection [1]. PTLD incidence depends on the type of organ transplantation, intensity of immunosuppressive therapy and EBV infection status prior to transplantation. In renal transplant recipients, PTLD is a rare complication (1.1% [2, 3] – 2% [1, 4]), more frequent in children than adults (1.2–10.1%) [2, 4] due to their greater likelihood of being naïve to EBV or cytomegalovirus (CMV) infection [5]. Mortality rates are often quoted at high levels, ranging from 50–70% [6].

Most cases of PTLD are associated with EBV infection, which increases EBV driven tumor formation in B-lymphocytes, based on poor immune control following IS agents. EBV-infected B-cells differentiate into memory B-cells or are killed by cytotoxic T-lymphocytes (CTL). Immunosuppressed transplanted patients have reduced CTL number, which may lead to proliferation of EBV infected B-cells, may allow reactivation, viral replication, EBV oncogene expression and malignant transformation of B-cells [4]. Additional stimuli are although required to promote the development of PTLD [7], however not all cases of PTLD are associated with EBV infection [4].

The current PTLD classification defined by World Health Organization (WHO) in 2008 is based on histological findings. Therefore, diagnosis of PTLD includes as gold standard histopathological tissue examination and additional studies of tissue sample (immunohistotyping, screening for presence of EBV and cellular clonality of tumors) [8]. Excisional biopsy is preferred but needle biopsy is also accepted [8, 9]. Classification of PTLD consists of: early lesions, monomorphic lesions, polymorphic lesions and Hodgkin’s lymphoma as described in Table 1 – adapted WHO Classification of PTLD (2008) [10, 11]. Monomorphic lesions in PTLD resemble non-Hodgkin’s lymphoma [4, 11], and are most frequent among PTLD forms (more than 70% of PTLD are monomorphic lesions) [11]. Considered rare (less than 5%) [11], Hodgkin’s-like lymphoma is not a part of the typical spectrum of PTLD following SOT [12].

Most common clinical features in PTLD are fever and lymphadenopathy. Other symptoms include extra lymph node involvement, abdominal symptoms (gastrointestinal disturbances, weight loss), hypotension or septic-like syndrome, infectious mononucleosis syndrome (sore throat, fatigue, anorexia, headache), hepatic or spleen enlargement, anemia, cytopenia, hemophagocytosis,
allograft dysfunction or central nervous system (CNS) related symptoms [13, 14]. An excisional biopsy, multiple needle biopsies or bone marrow biopsy may provide tissue for diagnosis.

| Table 1 – Adapted WHO classification of PTLD (2008) [11] |
|-----------------------------|-------------|-------------|
| **Histology** | **Frequency** | **EBV association** |
| **Early lesion** | | |
| • Plasmacytic hyperplasia; | 5% | 100% |
| • Infectious mononucleosis- | | |
| like PTLD | | |
| **Polyomorphic PTLD** | 15–20% | <100% |
| **Monomorphic B-cell PTLD** | >70% | 50% |
| • Diffuse large B-cell PTLD; | | |
| • Burkitt’s lymphoma; | | |
| • Plasmacytoma-like lymphoma; | | |
| • Other. | | |
| **Monomorphic T-cell PTLD** | <5% | 25–50% |
| • Peripheral T-cell lymphoma, not otherwise classified; | | |
| • Other. | | |
| **Classical Hodgkin’s lymphoma-type PTLD** | <5% | 100% |


Case presentation

We present the case of 18-year-old female patient from twins, both sisters being diagnosed at the age of 10 with nephropathitis (NPH), who underwent renal transplantation. This was the first simultaneous renal transplant from a deceased donor in twin sisters with CKD in Romania.

At the age of 10, she was admitted with polyuric-polydipsic syndrome. Microcysts were found in ultrasound examination in both kidney medullas, developing rapid progressive renal failure. She was tested negative for NPHP1 gene. Renal biopsy was not performed, as the family refused invasive procedure. Eighteen months from diagnosis, the patient was in stage V CKD and renal transplant was performed, together with her twin sister.

Postoperatory evolution was good with adjusted renal function. Immunosuppression treatment consisted of Tacrolimus, Thymoglobulin, Mycophenolate and Methylprednisolone.

During treatment, she developed moderate lymphopenia with values between 600 to 800 cells/mm³. After three months of lymphopenia and intensive immunosuppressive treatment, the patient was admitted with fever, stomatitis, lack of appetite and dysphagia. Lab tests showed leucopenia and anemia and increased creatinine levels. Although antigen viral tests were negative for CMV, herpes virus, EBV, treatment for this episode included Ganciclovir, reduction of Tacrolimus dosage and intravenous Immunoglobulin. Antiviral treatment was extended by three months of oral Valganciclovir and clinical evolution was favorable.

For the next six years, the evolution was marked by two episodes of urinary tract infection treated with antibiotics. Lymphocytes ranged between 1250 and 2200 cells/mm³ under Tacrolimus plus Mycophenolate treatment regimen.

After six years, the patient presented high fever, dizziness and lower abdominal pain. Lymphopenia was moderate, ranging between 400 and 600 cells/mm³ with severe combined deficit of B- and T-lymphocytes [B-lymphocytes count: 19 cells/mm³, T-lymphocytes count: 403 cells/mm³, CD4 lymphocytes: 127 cells/mm³, CD8 lymphocytes: 228 cells/mm³, natural killer (NK) lymphocytes: 296 cells/mm³] for a week, which lead to reducing Tacrolimus dosing to 50%. Abdominal magnetic resonance imaging (MRI) revealed retroperitoneal and periorbital lymph nodes up to 12 mm diameter. Viral antigens were negative for CMV, Toxoplasma and EBV and CMV viral load was undetectable. She had high inflammatory blood markers. EBV viral load was not performed at that time. She was treated with wide spectrum antibiotics and anti-fungals for 14 days with positive response and the patient was discharged. Lymph node biopsy was not performed as the family refused once again invasive diagnostic procedure.

One week without antibiotics lead to reappearance of high fever, dizziness, asthenia and lymphopenia. She was admitted and tested negative for PLEX-ID (detection of 780 bacteria and fungi), negative for tuberculosis. Atypical mycobacteria and viral infections were left to be blamed for clinical manifestations. Despite that, fever disappeared after three days of antibiotic treatment. We decided to perform Epstein–Barr viral load one month later. It showed high values (15 237 copies/μL) with no antibody response (EBV IgM and IgG negative). Oral Acyclovir was started and after one month, the EBV viral load (52 746 copies/μL) was increased. Clinical appearance had worsened by that time: constant fever, thymus hypertrophy, submandibular and cervical lymph nodes, pleurisy, ascites, pericarditis, anemia and jaundice had completed the clinical exam. Bone marrow aspiration revealed Reed–Sternberg cells, CD20-positive and EBV-encoded RNA (EBER) cells, suggestive of EBV infection. Microscopic urinary analysis indicated a complex immune process with intravascular erythropagocytosis, also consistent with viral infection.

Facing a progressive Epstein–Barr infection, Acyclovir was replaced by Ganciclovir, and immunosuppressive treatment reduced and changed to Sirolimus and steroid therapy.

Following this, clinical and serological values underwent mild improvement followed by rapid alteration of the patient’s clinical condition: jaundice secondary to lymph node compression on hepatobiliary tract, hepatomegaly (liver autopsy fragment showed atypical Hodgkin and Reed–Sternberg cells – Figure 1), splenomegaly, pericarditis and severe lymphopenia. All these led to unfavorable outcome within two weeks. Cause of death was classical Hodgkin’s lymphoma with lymphocyte depletion PTLD type, EBV-positive.

Morphological and immunohistochemical examination of abdominal lymph node revealed scattered atypical large cells with Hodgkin and Reed-Sternberg (HRS) morphology, CD15+/CD30+/CD40+, EBV-LMP1 (latent membrane protein 1) diffusely positive in the HRS cells, while EBNA2 (EBV nuclear antigen 2) is completely negative, both consistent with EBV latency II (Figures 2 and 3).
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Discussion

Renal-transplanted patients use immunosuppressant (IS) therapy to reduce the risk of graft rejection. There was an initial rise in incidence of PTLD after the extensive use of Cyclosporine [7]. Monitoring blood levels and reducing dosage accordingly led to decrease in incidence of PTLD [7]. No significant difference could be found in the incidence of PTLD following Tacrolimus compared to Cyclosporine regimens [15].

There are several risk factors for PTLD: EBV infection being an important one, found in about 60% of cases [16], use of drugs such as OKT3 (Muromonab) [17], Thymoglobulin, anticalcineurinics agents (Cyclosporine, Tacrolimus) [3, 17] and other viral infections (CMV, hepatitis C) [18]. Age less than 20, white race, negative EBV and CMV serology at the time of transplant represent risk factors for early PTLD [19], while age less than 20 or more than 50 years and Hispanic race are correlated with late PTLD [20].

Our patient had risk factors of developing PTLD after kidney transplant: age less than 20, Thymoglobulin treatment, intensive immunosuppression and negative EBV serology at the age of transplant.

PTLD therapy is based on antiviral agents (Acyclovir or Ganciclovir) and reducing IS agents. Antiviral agents can be effective on early or polymorphic lesions of PTLD but there is little data about their effectiveness on monomorphic disease [13]. There is not enough data about proven benefits when using intravenous immunoglobulin (IVIG), CMV-hyperimmune globulin in PTLD treatment [14].

Anti-B-lymphocyte antibodies (Rituximab) is mentioned to be used for inducing remission as monotherapy or in combination with chemotherapy for B-cell PTLD [21, 22], also with positive effects in CD20+ PTLD in high risk patients (multivisceral transplant, evidence of persistent or progressive PTLD in absence of allograft rejection) [14, 23]. Reduction in IS therapy can and may lead to remission in early disease [4], while chemotherapy can be an option for non-responding or highly aggressive PTLD [6], with serious side effects. Chemotherapy (low-dose Cyclophosphamide associated with steroid therapy) is preserved for progressive or persistent PTLD after IS reduction or Rituximab use, simultaneous allograft rejection or fulminant PTLD [14]. In pediatric PTLD, low dose chemotherapy (Cyclophosphamide and Prednisone) had good clinical response [24].

In our case, positive diagnosis was Hodgkin’s-like lymphoma PTLD, established afterwards, by autopsy of abdominal lymph node examination (previously refused by family). Treatment regimen was reducing IS therapy (Tacrolimus was changed to Sirolimus, Mycophenolate aborted and corticotherapy was started) and use of antiviral agents (Acyclovir at first and then changed to Ganciclovir). Further treatment lines were not applicable due to fragile clinical condition and fulminant evolution that led to unfavorable outcome.

In absence of effective treatment of PTLD, our focus must be on prevention. High risk of developing PTLD patients should be identified before transplantation by determining EBV status and detecting comorbidities such as CMV infection [25, 26]. EBV negative are found to have an increased risk of PTLD [25]. Using Valganciclovir prophylaxis after organ transplantation for six to 12 months is an effective method to prevent EBV/CMV infection. Adjusting the Valganciclovir dose monthly in pediatric
patients reduces the risk of under-dosage and therefore the incidence of EBV infection [27]. Aggressive supplemental IS should be used in biopsy-proven acute graft rejection. It is also important to establish standardized criteria for distinguishing early PTLD of graft rejection [9]. Hodgkin’s-like lymphoma PTLD is a rare subtype of PTLD, which often presents as late onset and is EBV-associated.

Twin kidney transplanted sister had also high detectable EBV viral load (262 000 copies/μL) with acute EBV infection [viral capsid antigen (VCA) IgM+, VCA IgG+, early antigen (EA) IgG+, EBNA IgG negative] but without any clinical features of PTLD. She underwent intravenous Ganciclovir therapy followed by six months oral Valganciclovir. IS reduction was performed. Global immune response in twin sister is normal (B-lymphocytes count: 193 cells/mm³, T-lymphocytes count: 1384 cells/mm³). She is in a follow-up program.

Conclusions

There is a must for routine testing for EBV infection in SOT patients in order to identify patients at risk of PTLD and apply early treatment. Even if EBV antigen detection is negative, EBV viral load should be mandatory. Also, immune system exploration should be done when EBV viral load is present. Frequent IS agents’ dose adjustment is important in keeping balance between EBV proliferation and graft rejection. Histopathological tissue examination is necessary for accurate diagnosis. The follow-up program of patients is needed in order to prevent, detect and treat such severe complications, preserving life.

Conflict of interests

The authors declare that they have no conflict of interests.

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