

Late Epstein-Barr virus-related post-transplant lymphoproliferative disorder with intestinal involvement in patient with chronic graft-versus-host disease after allogeneic hematopoietic stem cell transplantation

HEDDA KLOSTER HJELBAKK¹, MONIKA MAŁGORZATA ADAMSKA^{1,2*}, BARTOSZ MAŁECKI^{2*}, KATARZYNA BRZEŹNIAKIEWICZ-JANUS³, ANNA ŁOJKO-DANKOWSKA², LIDIA GIL²

¹Hematology Research Group, Poznan University of Medical Sciences, Poznan, Poland

²Department of Hematology and Bone Marrow Transplantation, Poznan University of Medical Sciences, Poznan, Poland

³Department of Hematology, University of Zielona Gora, Multi-Specialist Hospital, Gorzow Wielkopolski, Poland

* These authors contributed equally to this study.

Abstract

Post-transplant lymphoproliferative disorder (PTLD) is a rare, but severe Epstein-Barr virus (EBV)-driven disorder that manifest after hematopoietic stem cell transplantation (HSCT) or solid organ transplantation (SOT). This heterogenous disease may manifest as localized or disseminated, and clinical presentation may differ significantly. It may be difficult to early diagnose PTLD, as it may be misdiagnosed as infection or graft rejection. The majority of EBV-PTLD typically occurs within four months following HSCT, and almost all cases present within the first year. EBV-PTLD that manifests > 5 years is considered an exceedingly rare occurrence.

We describe a case of 66-year-old male, who was diagnosed with high-risk chronic lymphocytic leukemia (CLL). He underwent allogeneic HSCT from HLA-identical sister, and subsequently developed acute followed by chronic graft-versus-host disease, for which he was long-term treated with immunosuppressants. At 6 years following HSCT, the patient presented with life-threatening perforation of gut. Histological evaluation revealed diffuse large B cell lymphoma. Serum sample test showed positive EBV DNA and diagnosis of probable EBV-PTLD was done. After the treatment with rituximab, along with the reduction of immunosuppression, the patient achieved complete remission. Late onset EBV-PTLD after HSCT is extremely uncommon, and hardly described in literature.

Key words: hematopoietic stem cell transplantation, post-transplant lymphoproliferative disorder, late onset.

(Cent Eur J Immunol 2020; 45 (2):233-236)

Introduction

Post-transplant lymphoproliferative disorder (PTLD) is a heterogeneous group of diseases occurring after transplantation of either allogeneic hematopoietic stem cells (allo-HSCT) or solid organs (SOT). It is caused by iatrogenic suppression of T cell function, which results in uncontrolled neoplastic proliferation of lymphoid or plasmacytic cells. 60-70% of all PTLD cases are related to Epstein-Barr virus (EBV); however, PLTD after allo-HSCT is EBV-related in almost 100% of cases [1]. PTLD is a rare, but

serious complication that is linked to significant morbidity and mortality after transplant. The incident of PTLD after HSCT is 3.2%. The vast majority (96%) of these cases appear within the first year after transplantation, with the median time described as 2-4 months [2]. After SOT, the incident ranges from 0.80% to 20% [3]. The spectrum of clinical presentation varies from localized lesions to fulminant disseminated lymphoproliferation. EBV-PTLD often presents in a non-specific way with a mononucleosis-like illness including fever, fatigue, hepatosplenomegaly, and

Correspondence: Monika Małgorzata Adamska, Hematology Research Group, Poznan University of Medical Sciences, 84 Szamarzewskiego St., 60-569 Poznan, Poland, e-mail: mmadamskaa@gmail.com

Submitted: 14.08.2018; Accepted: 17.06.2019

lymphadenopathy [4]. Other symptoms may be present depending on the various organs involved.

The most important risk factor for the development of PTLD after allo-HSCT is the EBV serostatus of the recipient at the time of transplantation. The incidence of PTLDs in EBV-seronegative recipients is higher, as EBV infection is usually acquired from the donor [5-7]. Other principal risk factors include the degree of T cell depletion, HLA mismatch, splenectomy, second HSCT, cord blood transplantation, development of acute or chronic graft-versus-host disease (GvHD), rising EBV viral load, and treatment with mesenchymal stem cells [2, 8].

Late-onset (> 1 year) PTLDs after allo-HSCT are not well-defined [1], and the present case illustrates a patient who developed intestinal EBV-PTLD, 6 years after allo-HSCT.

Case report

The patient, a 66-year-old man was diagnosed with chronic lymphocytic leukemia (CLL), Rai stage II, in 2004. Because of stable and asymptomatic disease, the patient was treated with the “wait and watch strategy” for the first 4 years after diagnosis. In 2008, progression of the disease occurred. TP53 gene mutation was detected, and treatment with chemotherapy with COP-R regimen was applied. Because of resistant disease, treatment was changed to FCR (fludarabine, cyclophosphamide, rituximab) regimen. Later, the patient received rituximab alone, which resulted with an improvement. Due to high-risk disease, he was qualified to allo-HSCT. His donor was his 56-year-old HLA-identical sister. The procedure was performed in June 2011. The patient was conditioned with reduced intensity therapy based on fludarabine (150 mg/m²) and cyclophosphamide (2,500 mg/m²), after which he was transplanted with peripheral blood stem cells. Both donor and recipient were EBV-positive and CMV-positive prior to the transplantation. GvHD prophylaxis consisted of standard doses of cyclosporine A (CsA) (3 mg/kg/daily *i.v.*) and methotrexate (15 mg/m² in day +1, next 10 mg/m² in day +3, +6, +11 after allo-HSCT *i.v.*). Neutrophil recovery (absolute neutrophil count > 0.5 × 10⁹/l) occurred on +19 day after transplant. He was discharged from the transplant unit at +29 day in good general condition.

One month later, the patient presented with severe diarrhea and skin changes consistent with acute GvHD grade 2. Initially, treatment included CsA (75 mg twice daily *p.o.*) and steroids (prednisone 1 mg/kg/day). Due to the lack of improvement, mycophenolate mofetil (MMF) (1,000 mg *p.o.* twice daily) was successfully added. The disease progressed to chronic, severe GvHD, with extensive skin and mucous membrane involvement. The patient remained on combine drug immunosuppression and extracorporeal photopheresis (ECP), which resulted in the stabilization of lesions. During the post-transplantation period,

he was monitored with CMV-DNA and EBV-DNA according to published guidelines and transplantation center policy. Later, the EBV viral load was monitored because of GvHD and treatment with high-dose immunosuppressive therapy. He underwent yearly reassessment of general status, including chest radiogram, abdominal sonography, cardiology assessment, and ophthalmology assessment with detailed biochemistry.

Six years after allo-HSCT, the patient was urgently admitted to the emergency ward due to sudden, severe abdominal pain, nausea, and vomiting. Currently, he was still on immunosuppression (steroids, cyclosporine, ECP). Clinical examination revealed inaudible intestinal peristalsis, and abdominal rigidity – symptoms consistent with peritonitis. Computed tomography (CT) scan of the abdominal cavity demonstrated the presence of parenteral gas in the peritoneal cavity, which suggested gastrointestinal perforation. The blood panel of the patient at that time was as follows: WBC 8.1 G/l (N: 4-10); LYMPH 0.29 G/l (N: 0.6-4.0); LDH 257 U/l (N: 100-248). Lymphocyte subtype counts were not available. The patient was admitted to the surgery department, and an urgent laparotomy was performed with resection of a fragment of small intestine with one-time reconstruction of continuity. The histopathological assessment of the material excised during laparotomy revealed the presence of diffuse large B cell lymphoma (DLBCL), with positive expression of CD20, MUM1, BCL6, BCL2, LPX:1, and Ki67 in almost 100% of cells. Neither EBV markers, checkpoint inhibitor expression, nor EBER ISH was evaluated from the histopathological sample. Richter transformation could be excluded based on the complete remission of CLL with negative MRD (flow cytometry). The patient underwent surgery due to a life-threatening condition in a district hospital without consultation with transplantologists or hematologists.

CT scan of thorax, abdomen, and pelvis revealed no other lesion, and no enlargement of lymph nodes was noted. This resulted in the diagnosis of stage IE according to the modified Lugano classification from Ann Arbor. High levels of EBV DNA (5 × 10⁵ copies/ml) were detected in serum and diagnosis of probable intestinal EBV-PTLD was established. The patient had been continuously treated with immunosuppressive drugs since allo-HSCT. Therefore, a treatment consisted of the reduction of immunosuppression with weekly rituximab administration and monitoring EBV DNA-emia until normalization was prescribed. EBV DNA negativity was achieved after 3 doses of rituximab, and remission was confirmed by positron-emission tomography (PET-CT) scan. Currently, the patient is in good general condition, with stable chronic GvHD. Rituximab is planned to be given bi-monthly and maintained for 2 years in total. There is no uniform recommendation regarding duration of rituximab application in PTLD. Rituximab exhibits immunosuppressive activity and with prolonged administration, CsA, and steroid

dosage, the dosage may be reduced. Long-term use of rituximab may cause neutropenia in a small percentage of patients. However, in our patient, there were no proven infectious complications during the hospital stay or later apart from EBV reactivation.

Control PET-CT scan is planned every 6 months. Currently, 14 months after EBV-PTLD diagnosis, EBV DNA, and PET-CT scan remain negative.

Discussion

EBV-PTLD is a rare complication after HSCT. However, an increasing incidence is observed due to a growing number of procedures as well as longer survival of HSCT recipients. The incidence of PTLT after allo-HSCT varies from 11.2% in mismatched unrelated donor (MMUD), 4% in matched unrelated donor (MUD), 2.8% in mismatched family donor (MMFD), to 1.2% in matched family donor (MFD) [6, 8].

The presented patient underwent MFD allo-HSCT. He was predicted to have a high-risk of developing PTLT due to steroid-refractory acute GvHD and chronic GvHD requiring intensive immunosuppression [2].

Since EBV-PTLD usually occurs in the first year after HSCT, it is recommended to frequently monitor high-risk patients during the initial post-transplant period. According to recent guidelines, monitoring of serum EBV DNA-emia should be continued at least 4 months after HSCT, with once a week frequency [2]. Longer monitoring of EBV DNA is suggested for patients on treatment for acute, chronic GvHD. However, there is no strict recommendation for how long and how often EBV DNA should be monitored. In the described case, the patient was monitored according to the guidelines. EBV DNA was negative 4 months before EBV-PTLD occurred, but significantly increased at the time of presentation of complications.

All PLTD tumors, irrespective of histology, are potentially life-threatening. Early diagnosis of PTLT is challenging due to non-specific symptoms and confusion of its early forms with infection or graft rejection. Late-onset PTLT may be misinterpreted as a relapse of the underlying disease. With suspicion of PTLT, the careful clinical assessment is indispensable. The biopsy of enlarged lymph node and other sites should be performed with a histological examination, including EBER ISH and/or immunohistochemistry for viral antigens, and/or flow cytometry. If such invasive methods are impossible due to condition of the patient, the diagnostic approach should rely on non-invasive methods such as PET-CT/CT imaging combined with peripheral blood PCR with EBV viral load [2]. GI symptoms are non-specific but may suggest the presence of EBV-PTLD tumor in an immunosuppressed patient. Although, if the patient is receiving treatment with different drugs, an altered gastrointestinal pattern may occur. This may disguise several warning signs of the development

of PTLT, especially if they appear in the gastrointestinal tract. Therefore, like in the presented case, it is sometimes hard to detect PTLT before it presents in a potentially life-threatening way. The patient's history and numerous risk factors should always be of concern even in long-term allo-HSCT survivors.

Monomorphic PTLTs of B cell origin represent most of adult cases, with DLBCL identified as the most common subtype [9], as was the result of our patient.

The diagnosis of proven EBV-PTLD can be established with the detection of EBV nucleic acids or EBV-encoded proteins in the biopsy of tissue specimens, together with clinical symptoms and signs of the disease [4]. Probable EBV-PTLD is diagnosed in the presence of significant lymphadenopathy or other end-organ diseases, with high serum EBV DNA load and in the absence of other etiologic factors or established diseases, such as in presented case [4]. The lack of EBV immunostaining of the biopsy of patient's intestines is the greatest restriction of PTLT diagnosing. If done, the diagnosis of proven PTLT could simply be confirmed or the involvement of EBV could be excluded.

In order to overcome the risk of multi-organ impairment and potentially life-threatening condition, fast implementation of therapy remains crucial [2]. The management of strategy in EBV-PTLD after HSCT consists of prophylaxis, pre-emptive therapy, and treatment. The treatment of PTLT targets the reconstitution of T cell function and reduction of proliferating B cells. Therapies involve administration of rituximab, reduction of immunosuppression (RI), EBV-specific cytotoxic T lymphocytes (CTL), donor lymphocyte infusion (DLI), chemotherapy, and surgical interventions in life-threatening conditions [10]. Rituximab monotherapy once weekly, with simultaneous monitoring of EBV viral load is the first-line therapy and demonstrates positive outcomes in approximately 70% of patients [2]. There is no uniform recommendation for how long rituximab should be given in terms of PTLT. Rituximab can reduce the risk of both acute and chronic GVHD [9], but long-term usage may cause neutropenia in a small percentage of patients. RI, defined as a decrease of at least 20% of immunosuppressants doses combined with rituximab administration, is also recommended, and may improve the outcome in over 80% of cases [6, 11]. Second-line therapy, which consists of cellular therapy (DLI, CTLs) or chemotherapy should be implemented if the progression of the disease is observed, or in case of developed resistance to rituximab [12]. Surgical interventions remain necessary in cases of PTLT as a medical emergency. The response to therapy in EBV-PTLD is defined as a decrease in EBV DNA-emia at least 1 log₁₀ in the first week of treatment.

As mentioned in this case report, the patient was treated with RI combined with rituximab administration (weekly for 4 weeks). Consequently, a gradual decrease in EBV DNA-emia, with final negativity was observed.

At 3 months later, PET-CT revealed a remission. Currently, 14 months after EBV-PTLD diagnosis, rituximab is administered every 2 months, and is planned to be maintained for the next 10 months.

The prognosis for EBV-PTLD after HSCT improved with the implementation of novel treatment strategies. Until 2000, the mortality of 84.6% was reported [13]. Recently, with pre-emptive therapy, monitoring of EBV DNA and therapy with rituximab estimated mortality concern with one-third of patients [6].

Conclusions

The presented case illustrates that it may be necessary to extend EBV DNA monitoring in patients with high-risk disease or in patients that are treated with long-term immunosuppressant therapy. Despite the heterogeneous clinical manifestations of EBV-PTLD, clinicians should always consider its extremely rare late occurrence, and that it may present in a severe and potentially life-threatening manner. Among patients after allo-HSCT who undergo unexpected surgical intervention, the detection of EBV nucleic acids or EBV-encoded proteins in tissue specimens should be performed. Because of lacking data of the clinical manifestations of late onset of EBV-PTLD in HSCT patients, a detailed analysis should be performed.

The authors declare no conflict of interest.

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