

# Diagnosis and treatment of neoplastic post-transplant lymphoproliferative disorder following hematopoietic stem cell transplant in $\beta$ -thalassemia

## A pediatric case report

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### Abstract

**Introduction:** Post-transplant lymphoproliferative disorder (PTLD) is the most common form of lymphoproliferation in childhood and is associated with significant morbidity and mortality. In this report we reviewed the case of a pediatric patient who experienced PTLD after allogeneic hematopoietic stem cell transplantation (HSCT) from a human leukocyte antigen (HLA)-identical sibling.

**Methods:** The clinical characteristics, diagnosis, and treatment of PTLD after sibling HSCT in a 4-year-old boy with severe  $\beta$ -thalassemia was retrospectively reviewed.

**Results:** Medical records revealed the patient developed a fever and superficial lymphadenopathy and soft palate enlargement 8 months post-HSCT. Pathologic diagnosis indicated non-Hodgkin lymphoma (B-cell type), which resulted in a reduced dose of immunosuppressant and the initiation of chemotherapy (administered according to the BFM95 protocol for 2 courses; 4 courses of rituximab therapy was also administered). Currently, the patient has been disease-free for over 3 years. There are no specific guidelines for the treatment of PTLD. The status of stem cell implantation after transplantation, and graft versus host disease should be evaluated jointly, and rituximab therapy and chemotherapy with BFM-95 may be used for treatment of pediatric PTLD after HSCT.

**Conclusion:** The current case represents a unique opportunity to review a pediatric patient with  $\beta$ -thalassemia. The successful treatment of post-transplant non-Hodgkin B lymphoma may help other physicians in the management of similar pediatric cases.

**Abbreviations:** EBV = Epstein–Barr virus, GVHD = graft versus host disease, HLA = human leukocyte antigen, HSCT = hematopoietic stem cell transplantation, PTLD = post-transplant lymphoproliferative disorder.

**Keywords:**  $\beta$ -thalassemia, chemotherapy, hematopoietic stem cell transplantation, post-transplant lymphoproliferative disorder

## 1. Introduction

Hematopoietic stem cell transplantation (HSCT) is an effective strategy to cure severe  $\beta$ -thalassemia in children with a demonstrated success rate of up to 90%; however, the source of hematopoietic stem cells (HSCs) may affect short-

and long-term complications, and management remains a great challenge for clinicians. After HSCT, post-transplant lymphoproliferative disorders (PTLDs), especially Epstein–Barr virus (EBV)-positive PTLD may occur with current treatments offering varying levels of efficacy.

PTLD is the most common form of lymphoproliferation in childhood and is associated with significant morbidity and mortality. There is no standard protocol for treatment of PTLD. Current treatment options include rituximab and chemotherapy but are associated with added immunosuppression and only lead to resolution in about 70% of patients.<sup>[1]</sup> Strategies used for the treatment of PTLD aim to inhibit viral replication, control hyperplasia of B cells, and improve surveillance of memory cytotoxic T cells. Corresponding chemotherapy is recommended in cases of tumor-related PTLD. In addition, treatment with monoclonal antibody, transfusion of donor lymphocytes, cytokine therapy, cellular immune therapy, and genetic therapy may also be applicable.<sup>[2]</sup> The use of rituximab, low-dose cyclophosphamide, and dexamethasone in the treatment of CD20+ EBV-related PTLD in children after solid organ transplantation has been reported.<sup>[3]</sup> EBV-specific T lymphocytes have shown promising results in the treatment of EBV-positive PTLD. Current research is focusing on increasing the efficacy of EBV-specific T-lymphocytes in the presence of immunosuppression.<sup>[1]</sup> Herein, we report a case of PTLD after HSCT in a child with severe  $\beta$ -thalassemia.

Editor: N/A.

This work was supported by the projects from Medical Scientific Research Foundation of Guangdong Province, China (no. B2010273), Medical and Health Technology Project of Guangzhou (no. 20142A011017), and Medical and Health Technology Project of Guangzhou (no. 2009-YB-068).

The authors report no conflicts of interest.

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Medicine (2017) 96:52(e9055)

Received: 6 November 2017 / Accepted: 13 November 2017

<http://dx.doi.org/10.1097/MD.0000000000009055>

## 2. Clinical information

A 6-month-old male was admitted to our hospital in June 2009 due to progressive psoriasis. A diagnosis of severe  $\beta$ -thalassemia (genotype CDS41-42/CDS41-42) was made at 1 year of age. Shortly after diagnosis, the patient was treated with periodic blood transfusions, and iron chelation therapy was administered after 10 blood transfusions. At 3.5 years of age (June 2013) the patient received a sibling HLA-identical HSCT of  $7.846 \times 10^8$ /kg of mononuclear cells and  $6.55 \times 10^6$ /kg of CD34+ cells from peripheral blood stem cells (Day 0).

The patient was negative for CMV-IgM, the EBV-DNA was  $<500$  cps/mL, and negative for human parvovirus B19-IgM, hepatitis A-IgM, hepatitis C-IgM, and human immunodeficiency virus (HIV) antibodies. A syphilis screening test was also negative. The sibling donor was also negative for CMV-IgM, EBV, human parvovirus B19-IgM, hepatitis A-IgM, and hepatitis C-IgM.

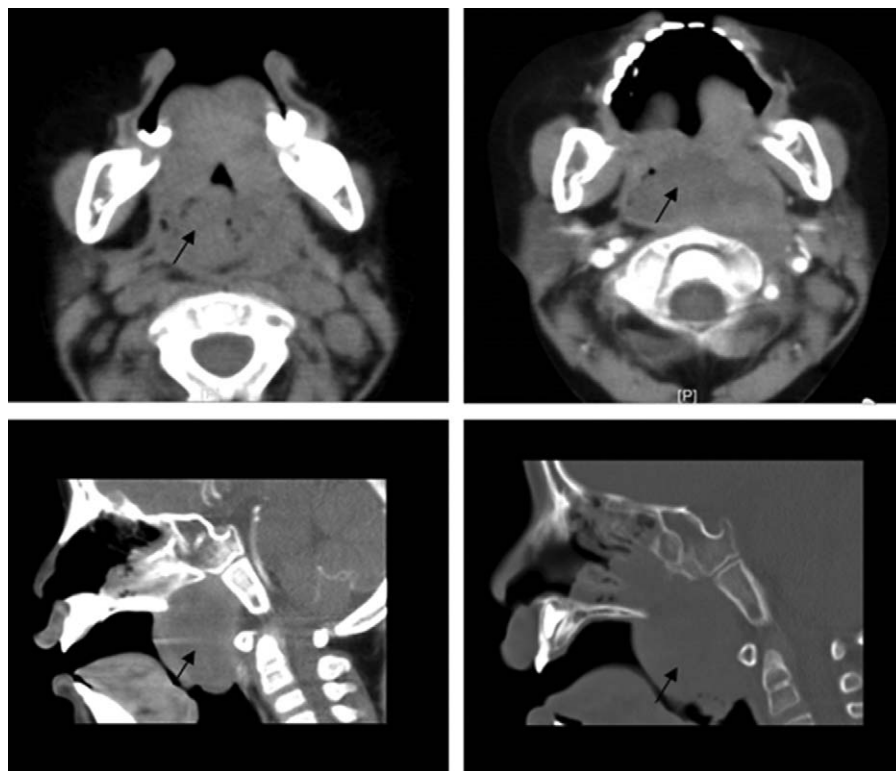
Pre-treatment was initiated 45 days before HSCT and consisted of the following: 30 mg/kg/d of hydroxyurea (Hu) (-45d to -12d); 20 mg/m<sup>2</sup>/d of Fludara (-17d to -13d); 0.8 mg/kg of busulfan (Bu) (q6h; -9d to -6d); 50 mg/kg/d of cyclophosphamide (-5d to -2d); and 2.5 mg/kg/d of horse anti-human thymocyte immunoglobulin (ATG; Pfizer ATGAM) (-4d to -2d). The protocol for prevention of graft versus host disease (GVHD) included administration of cyclosporine A (3 mg/kg/d, beginning on -5d) and MTX (+1d, 15 mg/m<sup>2</sup>; +3d, +6d, +11d, 10 mg/m<sup>2</sup>). The dosage of cyclosporine A was adjusted based on blood serum concentration to 150 ng/mL.

Bone marrow implantation was successfully completed at +15d. Chimera detection was confirmed at +18d, 3 days after

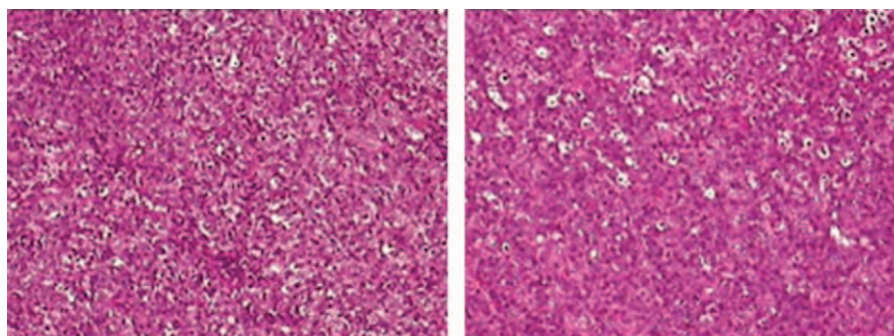
bone marrow implantation. The proportion of donor chimera was reduced from 95% to 79% considering the patient was dependent on blood transfusions. Lymphocyte transfusion was initiated on +162d, and a total of  $6.3 \times 10^7$ /kg donor lymphocytes were transfused. Upon an increase in the proportion of donor chimera to 83% the patient was no longer dependent on blood transfusions. Administration of cyclosporine and methylprednisolone were continued to prevent GVHD, and the concentration of cyclosporine was maintained at  $<100$  ng/mL.

Eight months after HSCT the patient developed nasal obstruction and cervical lymph node enlargement, as well as soft palate swelling and purulent secretions. EBV-DNA detection indicated  $5.09 \times 10^3$  cps/mL. Despite an anti-infective and focal symptomatic treatment, the nasopharyngeal mass progressively enlarged and the dyspnea worsened. The patient was then transferred to the pediatric intensive care unit (ICU) and received assisted breathing via mechanical ventilation. A CT scan showed that the pharyngeal airway was significantly stenotic. Upon further examination, the purulent secretions showed abnormalities (Fig. 1). A malignant nasopharyngeal tumor was suspected by physicians in the PICU and the Departments of Respiratory Diseases and Otorhinolaryngology. A biopsy of the nasopharyngeal mass was performed under general anesthesia, which revealed small, round tumor cells that displayed diffuse hyperplasia, and cellular atypia. The cytoplasm was scant, the nucleus was round, and the chromatin was thick. One to 2 nucleoli were clearly visible in each cell, and nuclei division was active (Fig. 2). Non-Hodgkin lymphoma (B-cell type) was considered.

Immunohistochemistry of the nasopharyngeal tumor showed the following features: PAX5+; CD20+; diffusely CD3+; CD10-; TdT-; MPO-; CD30/ALK/LMP-; and the proportion



**Figure 1.** The CT scan indicated posterior pharyngeal wall thickening, iso- or hypo-intensities, largest width = 3 cm, and length = 5.2 cm. The plain CT value was 39 HU. There were bubble shadows. An enhanced CT scan showed irregular peripheral enhancement at the periphery, and there was no obvious central enhancement.



**Figure 2.** H&E staining. Under a light microscope, the round tumor cells displayed diffuse hyperplasia, and cellular atypia was apparent. The cytoplasm was scant, the nucleus was round, and the chromatin was coarse. There were 1 to 2 nucleoli in each cell, and the mitotic figures were not reactive.

of Ki67-positive cells was 80%. Upon pathologic examination, the tissues were partially necrotic under a light microscope, infiltration of lymphocyte-like cells with uniform morphology was detected in a portion of the tissues, nuclear fission was observed, in situ hybridization showed EBERs(+), from phenotype, possibility of lymphoma was considered. Immunohistochemistry showed: LCA (+); Bcl2(+); PAX5(+); CD20(+); CD79a(+); CD43(+); Mum1(+); focally CD30+; ALK(-); CD56(-); CD21(-); CD10/CD5/CD3/GrB/TIA-1/Bc16(-); and the proportion of Ki67-positive cells was approximately 70%. B-cell lymphoma IgH rearrangement was positive. Repeated morphologic examinations of bone marrow cells showed proliferative characteristics; primitive and naive granulocytes accounted for 6.5% to 11% of the bone marrow cells. Flow cytometry failed to identify a naïve cell population. Thus, nasopharyngeal non-Hodgkin lymphoma was considered (B-cell type; stage II).

After a diagnosis of non-Hodgkin lymphoma, the dose of cyclosporine and methylprednisolone was tapered. Additionally, ganciclovir was administered at 5 mg/kg (q12h) for 2 weeks, and chemotherapy was initiated at +270d using the BFM-95 B protocol, in which dexamethasone (10 mg/m<sup>2</sup>/d) and cyclophosphamide (200 mg/m<sup>2</sup>/d) were administered on d1-5 and d1-2, respectively. After a 2-day treatment break, cyclophosphamide (350 mg/m<sup>2</sup>/d, d1), THP (25 mg/m<sup>2</sup>/d, d1-2) and dexamethasone (10 mg/m<sup>2</sup>/d, d1-7) were administered; anti-infective, hydration, and alkalization therapies were initiated at the same time. After treatment, the soft palate mass gradually shrunk, the enlarged neck lymph nodes gradually decreased in size, and mechanical ventilation was discontinued. A PET/CT scan showed the following: nasopharyngeal foci with high metabolism after chemotherapy for nasopharyngeal lymphoma, and residual tumor was considered; and several nodules with high metabolism were observed in the posterior left orbit, left nasopharyngeal space, and left neck, and lymphoma invasion was considered. During the treatment, re-examination of the EBV+CMV-DNA titer was reduced to <500 cps/mL, and the proportion of chimeric cells was 83%.

Rituximab was administered at 375 mg/m<sup>2</sup>/d at +284d and +298d. Course A chemotherapy was administered at +316d, according to the BFM95 protocol (dexamethasone 10 mg/m<sup>2</sup>/d, d1-5; VCR 1.5 mg/m<sup>2</sup>/d, d1; IFO 800 mg/m<sup>2</sup>/d, d1-5; Ara-C 150 mg/m<sup>2</sup>, q12h, d4-5; VP16 100 mg/m<sup>2</sup>/d, d4-5; and MTX 1 g/m<sup>2</sup>/d, d1) and an intrathecal injection was given on day 1 with MTX +Dex+Ara-C, with hydration and alkalization therapies.

Course B chemotherapy was administered at +342d, according to the BFM95 protocol (dexamethasone 10 mg/m<sup>2</sup>/d, d1-5; VDS

3 mg/m<sup>2</sup>/d, d1; CTX 800 mg/m<sup>2</sup>/d, d1-5; THP 30 mg/m<sup>2</sup>, q12h, d4-5; VP16 100 mg/m<sup>2</sup>/d, d4-5; and MTX 1 g/m<sup>2</sup>/d, d1) and an intrathecal injection was given on day 1 with MTX+Dex+Ara-C.

The patient had an increased frequency of defecation at +343d, with dark green stool with mucus and white membrane-like substances. A white pseudomembrane was noted on the posterior wall of the mouth and pharynx, as well as a systemic rash, some of which was coalesced. The total bilirubin, direct bilirubin, and indirect bilirubin were significantly increased. According to the Glucksberg criteria, he was diagnosed with grade III GVHD. Methylprednisolone, cyclosporine, and mycophenolate mofetil were administered for immune suppression—gamma globulin was administered intravenously. Gastrointestinal food intake was restricted, and parenteral nutritional support was initiated. As a result, the frequency of defecation was reduced, stool characteristics normalized, and the white pseudomembrane in the mouth disappeared. In addition, the systemic rash and jaundice were attenuated, the abdominal pain resolved, and food intake returned to normal. Blood test and biochemical examine at +376d were normal (cyclosporine concentration of 208.00 ng/mL). A CT scan showed that the nasopharyngeal tumor had disappeared.

Rituximab was intravenously infused at 375 mg/m<sup>2</sup>/d at +389 d. A PET/CT scan showed no lesion. Twenty-six months after HSCT, pharmacotherapy was discontinued. The patient has been disease-free for over 3 years. There were no abnormalities upon physical examination or further laboratory testing.

### 2.1. Ethics approval and consent to participate

Our institutional review board was waived due to the retrospective nature of the study. All included patients gave their informed consent.

## 3. Discussion

In this report we reviewed the case of a pediatric patient who experienced PTLD after allogeneic HSCT from an HLA-identical sibling. PTLD is a complication of HSCT and other solid organ transplantations, and is the most common malignancy related to transplantation in children.<sup>[4]</sup> PTLD is often acute and disseminated, progresses rapidly, and has an uncertain prognosis. The incidence of PTLD is affected by the type of primary diseases and donors, HLA condition, pre-treatment protocol, and measures for the prevention of GVHD in allogeneic HSCT.<sup>[5]</sup> The overall prevalence of PTLD after allo-HSCT has been

reported to be between 1% and 2%, and as high as 24% after T-cell-free allo-HSCT. In children, the incidence of PTLD is significantly higher than adults, and >90% of PTLD is related to EBV infection.<sup>[6]</sup> To date, no study has reported tumor-related PTLD in children with thalassemia after HSCT, although 2 patients with major and intermedia  $\beta$ -thalassemia complicated with Hodgkin lymphoma and chronic myelogenous leukemia during the course of the disease have been described.<sup>[7]</sup>

The current patient was diagnosed with stage 2 monomorphic PTLD (non-Hodgkin lymphoma, B-cell type). The typical pathology of PTLD is the presence of a large number of plasma cell-like B cells, a few T cells among lymphocytes, and focal necrosis.<sup>[8]</sup> The WHO classifies PTLD as follows<sup>[9]</sup>: early manifestations of PTLD; polymorphic PTLD; monomorphic PTLD; and Hodgkin lymphoma (HL)-like PTLD. After transplantation with a solid organ, monomorphic PTLD may be observed in 72% of children, and 97% of monomorphic PTLD originates from B cells.<sup>[10]</sup>

The common clinical manifestations of PTLD include fever, lymph node enlargement, hepatomegaly, splenomegaly, and pharyngitis. PTLD with involvement of the central nervous system may cause seizures, gait instability, and altered consciousness. PTLD usually progresses rapidly and may cause multiple organ failure or even death soon after diagnosis. However, it has been reported that PTLD is confined to local tissues 1 year after HSCT, suggesting a slow progression.<sup>[11]</sup>

It has been reported that the preemptive use of rituximab and EBV-CTL can reduce the risk of death due to EBV-PTLD in the setting of allogeneic HSCT. Therapy with either of these modalities can also reduce the risk of death due to PTLD, yet overall success rate was lower than after preemptive use of the therapies. Reduction of immunosuppression could be contemplated, but the risk of GVHD has to be taken into account. Other therapeutic strategies such as DLI, chemotherapy, or use of antiviral agents has at best a limited place in the management of PTLD.<sup>[12]</sup>

#### 4. Conclusions

The present patient was diagnosed with EBV-related lymphoma after HSCT, and subsequent reduction in the dose of immunosuppressants caused severe GVHD. Thus, the use of immunosuppressants should be adjusted according to timely evaluation of

clinical conditions. Early chemotherapy with low drug doses was helpful for the control of tumor-related complications, which may save time during subsequent treatments.

The current case represents a unique opportunity to review a pediatric patient with  $\beta$ -thalassemia, as most reported cases are adults. The successful treatment of post-transplant non-Hodgkin B lymphoma may help other physicians in the management of similar pediatric cases.

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