A case of Epstein Barr virus-related post-transplant lymphoproliferative disorder after haploidentical allogeneic stem cell transplantation using post-transplantation cyclophosphamide

Epstein Barr virus-related lymphoproliferative disorder (EBV-PTLD) is a rare but potentially fatal complication of allogeneic hematopoietic cell transplantation (HCT). Incidence of EBV-PTLD varies from 0.45% in matched sibling donor HCT to 29% in T-cell depleted matched unrelated donor HCT.[1-2] Risk factors include unrelated or HLA-mismatched donors, T-cell depleted grafts, ATG-based conditioning, umbilical cord recipients, donor-recipient EBV serostatus mismatch, and splenectomy.[3] In higher-risk transplants, prospective monitoring of EBV viremia by quantitative PCR assay is recommended.[4]

Ex vivo T-cell depleted haploidentical HCT (haploHCT) is considered high risk for EBV-related PTLD with an incidence up to 25%.[3] In pediatric HCT, preemptive rituximab therapy is a reasonable, albeit partially effective, option in high-risk populations, but second-line therapy (*e.g.* chemotherapy, cytotoxic T lymphocytes) may be necessary.[5] Post-transplant cyclophosphamide (PTCy)-based haploHCT, is considered low risk for EBV-PTLD despite numerous studies showing higher rates of other infectious complications compared to HLA-matched transplants.[6-7] Viral reactivation, particularly cytomegalovirus (CMV) and BK virus, are common and at least one documented infection of any source has been reported in 70-89% of patients.[8,9] Interestingly, in none of these studies was EBV-PTLD observed. Furthermore, the Johns Hopkins group retrospectively evaluated EBV-PLTD incidence after PTCY-based HCT.[10] There were no EBV-PTLD cases in 762 HCT recipients of HLA-haploidentical, matched-related, and matched- or one antigen mismatched-unrelated grafts at 1-year follow-up. Fourteen patients received ATG-based conditioning.[11] Hypotheses for low EBV-PTLD risk include the destruction of donor and host EBV-infected B cells, with relative sparing of EBV-specific memory T cells and rapid immune reconstitution following PTCy.[10]

The increasing utilization of haploHCT highlights the need for appropriate infectious monitoring and treatment.[12] Given the low-to-absent rates of EBV-PTLD reported, routine EBV monitoring in PTCY-based haploHCT appears unnecessary. However, we describe a first case of life threatening EBV-PTLD after PTCY-based haploHCT, suggesting a potential role for EBV monitoring.

A 51-year-old male with severe aplastic anemia (SAA) who previously failed immunosuppressive (IS) therapy with horse and rabbit ATG/tacrolimus and eltrombopag presented for alloHCT. His ANC, hematocrit (HCT) and platelets were 0.32 K/uL, 20.1%, and 8 K/uL, respectively at presentation. He was transfusion-dependent and required antibiotics for neutropenic prophylaxis. He underwent PTCY-based reduced intensity conditioning (RIC) haploHCT from his 16-year-old EBV-seropositive daughter due to the lack of matched sibling or unrelated donors.

His conditioning regimen comprised of rabbit ATG, fludarabine, cyclophosphamide and total body irradiation (TBI) per the Johns Hopkins SAA regimen.[13] A bone marrow graft with $3.63x10^6$ CD34 cells/kg and $2.21x10^8$ TNC/kg was infused on day 0. Graft-*versus*-host disease prophylaxis consisted of PTCy on day +3 and +4, followed by tacrolimus and mycophenolate mofetil (MMF). His hospital course was relatively uncomplicated. He engrafted white blood cells on day 32, although he remained transfusion-dependent for red blood cells and platelets for several weeks.

Prospective virological monitoring during the post-transplant period including weekly CMV DNA (IU/mL) levels and EBV blood PCR (IU/mL) levels (Figure 1). 99 days post-transplant, EBV viremia was noted (1,300 IU/mL). Two days later (day +91), he urgently presented to clinic with a new onset fever (102°F), sore throat, and bilateral cervical lymphadenopathy. A neck computerized tomography (CT) scan demonstrated right posterior cervical lymphadenopathy (largest node 1.1x1.4 cm), prominent tonsillar tissue, and multiple sub-centimeter lymph nodes. His EBV level had increased >10-fold to 13,100 IU/mL and he was admitted with clinical concern for EBV-PTLD. The following day, with a repeat EBV level of 33,000 IU/mL, he received rituximab 375 mg/m^2.

Despite rituximab, on day +96 he progressed with an increasing sore throat, dysphonia, rapidly enlarging cervical lymphadenopathy, new supraglottic edema and airway narrowing on direct laryngoscopy. He received

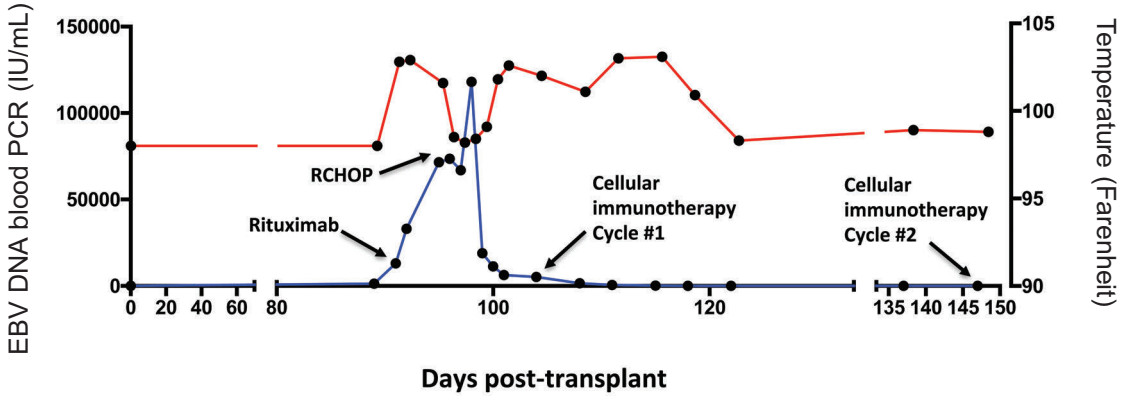

Figure 1. Results of virological monitoring of Epstein-Barr virus (EBV) levels (blue) and corresponding daily maximum temperature (red) during the first 150 days post-transplant.

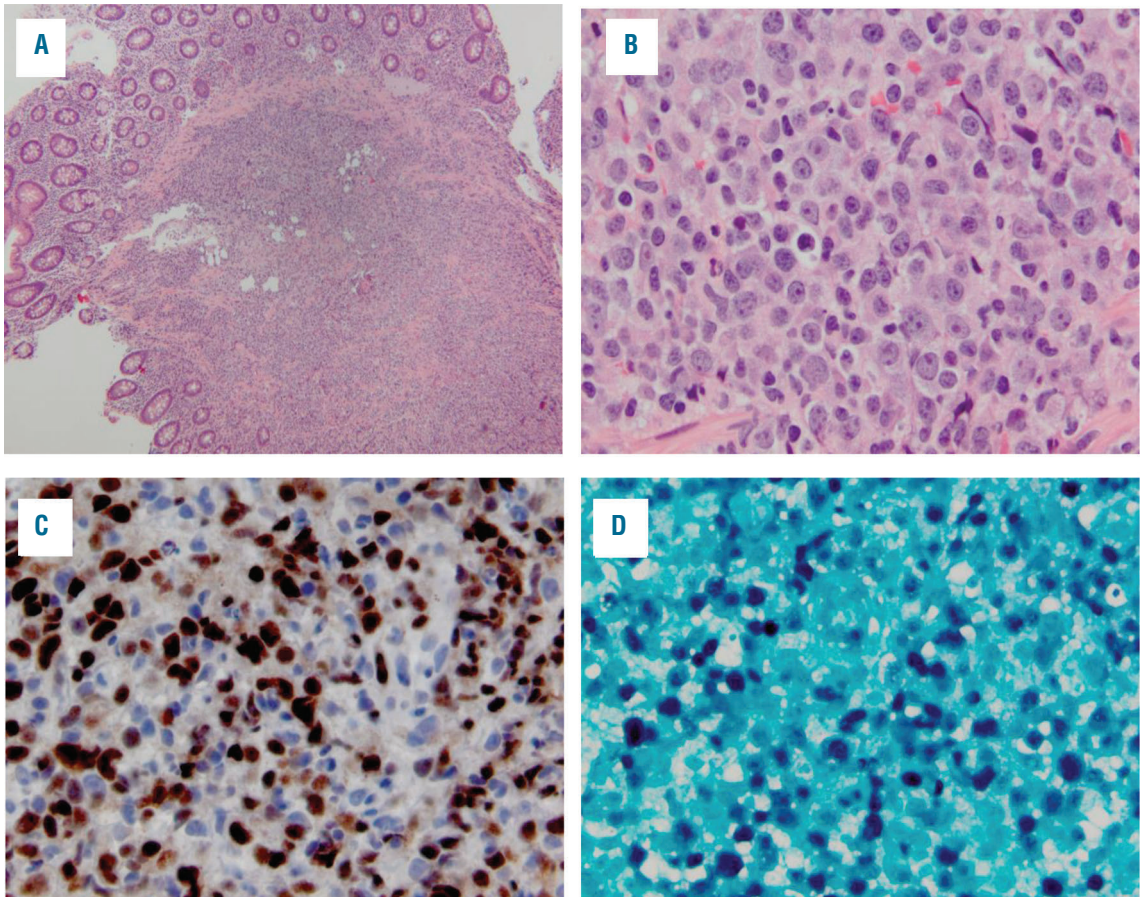

Figure 2. Histology and immunochemistry of the colonic biopsy. (A) Hematoxylin and eosin (H&E) staining at 40x magification and (B) 400x. (C) Immunohistochemistry at 400x showing BSAP-positive B cells with patchy positivity for CD20, in keeping with prior rituximab therapy (not shown). (D) Epstein-Barr virus (EBV) encoded RNA (EBER)+ at 400x. Not shown: BCL-2+ (90%), CD 30+ (50%), MUM1 +, CD 10 -, BCL-6 -, in keeping with an activated B-cell phenotype. MYC was positive in a subset of cells but overall less than 40%, and the Ki67 index was high at 80%.

emergent dexamethasone 40 mg intravenously for two days. He also noted worsening diarrhea, and a CT scan indicated rectal and cecal intramural thickening with possible colitis. Colon and left neck lymph node biopsy were undertaken (on day +95 and +96, respectively) and resulted on day +97 with pathology consistent with EBV-PTLD, monomorphic B-cell type (Figure 2). He initiated RCHOP chemotherapy plus rapid IS taper on day +97. His EBV viral load was 66,900 IU/mL on day 1 of RCHOP, peaked at 118,000 IU/mL the following day, and serially declined thereafter, with clinical improvement in his lymphadenopathy, diarrhea and sore throat. Due to the severity of his clinical status, he enrolled in a clinical trial and received three cycles of cellular immunotherapy. The first of these was initiated on day 104 with an EBV blood PCR level of 5,220 IU/mL.

His fevers persisted through chemotherapy, multiple immunotherapy infusions, reduction in lymphadenopathy and declining EBV levels to 1,540 IU/mL by day +108. With a chest CT scan showing a progressing left upper lobe nodular infiltrate, he was empirically treated with posaconazole and G-CSF. He became afebrile on day +118 with an undetectable EBV DNA level and resolution of lymphadenopathy. Due persistent pancytopenia post chemotherapy, a CD34 selected haploidentical stem cell boost was infused on day 133, with good recovery in his pancytopenia a few weeks later.

A PET/CT scan (day +137) was consistent with a partial response, demonstrating markedly decreased tonsillar and submandibular lymph nodes and a modest improvement in FDG-avid parotid glands. At the most recent follow-up (day +168), fevers, lymphadenopathy, tonsillar enlargement, and diarrhea had resolved. Another follow-up PET/CT scan showed complete response. He is being considered for low-dose DLI from his EBV seropositive stem cell donor for consolidation of EBV control.

Here, we present the first reported case of EBV-related PTLD after PTCY-based haploHCT. Traditional risk factors consisted of ATG conditioning and a haploidentical graft. However, PTCy-based haploHCT has been associated with very low to absent rates of EBV PTLD.[10] Several prospective studies have reported on EBV reactivation after PTCy-based haploHCT for SAA, yet none went on to develop EBV-PTLD. Dezern *et al.* prospectively studied 16 patients undergoing RIC HCT for refractory SAA, all receiving ATG-based conditioning and PTCy-based graft-*versus*-host disease prophylaxis. Only one patient developed EBV reactivation; however, the viral load did not require therapy and returned to undetectable within five weeks.[13] This result is in accordance with others, such as the prospective study by Clay *et al.* that reported on eight SAA patients undergoing RIC PBSC haploHCT with PTCy. EBV viremia was detected in 5 of 8 (62.5%)

patients however there was no EBV-related PTLD.[14] Finally, a retrospective multicenter study by Esteves *et al*. analyzed the outcomes of 16 SAA patients undergoing ATG-based RIC haploHCT and PTCy, with no EBV PTLD.[15]

Prospective virological monitoring allowed for early detection of EBV reactivation for our patient. However, based on prior data showing low-absent rates of PTLD in PTCy-based haploHCT, it could be argued that routine EBV monitoring was not warranted. Due to EBV surveillance, the patient received prompt treatment with rituximab but required second-line RCHOP therapy for explosive progression, with a rapid decrease in in EBV-DNA of over 20-fold (118,000 IU/mL to 5,220 IU/mL). Cellular immunotherapy was added due to disease severity, and the EBV viremia cleared two weeks later.

In conclusion, this case demonstrates that patients undergoing PTCy-based haploidentical HCT, especially those receiving ATG-based conditioning, remain at risk for life-threatening EBV-PTLD. We suggest routine monitoring of EBV-viremia reactivation, especially if there are other risk factors for PTLD.

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