Annals of Hematology (2019) 98:2163–2177
https://doi.org/10.1007/s00277-019-03742-7

ORIGINAL ARTICLE

Risk factors and clinical outcomes of Epstein–Barr virus DNAemia and post-transplant lymphoproliferative disorders after haploidentical and matched-sibling PBSCT in patients with hematologic malignancies

Xiao-Ning Gao[1] · Ji Lin[2] · Li-Jun Wang[1] · Fei Li[1] · Hong-Hua Li[1] · Shu-Hong Wang[1] · Wen-Rong Huang[1] · Chun-Ji Gao[1] · Li Yu[1] · Dai-Hong Liu[1]

Received: 4 March 2019 / Accepted: 15 June 2019 / Published online: 26 June 2019

Abstract

In allogeneic hematopoietic stem cell transplantation recipients, reactivation of Epstein–Barr virus (EBV) can cause post-transplantation lymphoproliferative disorder (PTLD), which may rapidly progress to multiorgan failure and even death. Development of EBV PTLD correlates very closely with use of anti-thymocyte globulin (ATG) and type of transplant. To assess the incidences and clinical features of EBV DNAemia and PTLD in the setting of stem cell transplantation using unmanipulated G-CSF-primed allogeneic peripheral blood stem cells as graft, we performed a retrospective analysis of stem cell transplantation from HLA-matched sibling donors (MSD-SCT, $n = 90$) or HLA-haploidentical related donors (HID-SCT, $n = 110$) in patients with hematological malignancies. All of HID-SCT recipients and 27.8% of MSD-SCT recipients received an ATG-containing conditioning regimen. One-year cumulative incidence of EBV DNAemia was 44.1%, ranging from 4.8% in MSD-SCT recipients not using ATG to 20.0% in MSD-SCT recipients using ATG, and 73.7% in HID-SCT recipients. Risk factors for EBV reactivation included use of ATG ($p = 0.008$), male donor ($p = 0.034$), and cytomegalovirus DNAemia ($p < 0.001$). One-year incidence of EBV PTLD was 11.9%, ranging from 1.8% in recipients of MSD-SCT not using ATG to 4.4% in recipients of MSD-SCT using ATG, and 23.5% in recipients of HID-SCT. Risk factors for PTLD after HID-SCT included in fludarabine-containing conditioning regimen ($p = 0.010$), cytomegalovirus DNAemia ($p = 0.036$), and patient's age < 40-yr ($p = 0.032$). Two-year non-relapse mortality was higher for patients with EBV DNAemia than those without EBV DNAemia (35.8% vs. 15.3%, $p = 0.002$). One-year relapse-free survival and overall survival among patients with PTLD were 40.2% and 44.9%, respectively, as opposed to 63.4% and 68.4% among patients without PTLD (both $p < 0.05$). In multivariate analyses, EBV DNAemia predicted a lower risk of relapse ($p = 0.025$), while PTLD was a marginally significant predictor of relapse ($p = 0.092$). This study identified patients at risk of EBV reactivation and PTLD after unmanipulated allogeneic peripheral blood stem cell transplantation.

Keywords Epstein–Barr virus · Post-transplantation lymphoproliferative disorder · Allogeneic peripheral blood stem cell transplantation · Haploidentical · Matched sibling

Xiao-Ning Gao, Ji Lin and Li-Jun Wang contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00277-019-03742-7) contains supplementary material, which is available to authorized users.

✉ Xiao-Ning Gao
gaoxn@263.net

✉ Dai-Hong Liu
daihongrm@163.com

[1] Department of Hematology, Chinese PLA General Hospital, Medical School of Chinese PLA, 28 Fuxing Road, Beijing 100853, China

[2] Institute of Basic Medicine, Chinese PLA General Hospital, Medical School of Chinese PLA, 28 Fuxing Road, Beijing 100853, China

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a well-established and effective therapy for a variety of hematological disorders. In allo-HSCT recipients, reactivations of Epstein–Barr virus (EBV), being one of the most common viruses in humans, can cause life-threatening complications such as post-transplantation lymphoproliferative disorder (PTLD) or end-organ diseases (encephalitis/myelitis, pneumonitis, hepatitis, or hemophagocytic lymphohistiocytosis). The incidence of EBV DNAemia and EBV PTLD varies between HSCT centers. The overall incidence of EBV DNAemia ranges from 0.1 to 63%, which depends on type of transplantation, sensitivity of EBV quantitative assay, and defined cut-off value of DNAemia [1–5]. According to a recent study [1], the overall incidence of EBV PTLD is 3.2% among allo-HSCT recipients, ranging from 1.2% in human leukocyte antigen (HLA)–matched sibling donor (MSD) to 2.9% in HLA-haploidentical related donor (HID), 4.0% in HLA-matched unrelated donor, and 11.2% in HLA-mismatched unrelated donor recipients. Several risk factors may be associated with development of EBV PTLD after allo-HSCT, including T cell depletion of the donor bone marrow, use of anti-thymocyte globulin (ATG), use of reduced intensity conditioning, HLA-mismatched related or unrelated donor, pretransplant splenectomy, patient's age ≥ 50 years, and acute and chronic graft-versus-host disease (GVHD) [6, 7]. During the past two decades, data have shown an increased incidence of EBV PTLD and significant association with mortality [8]. This is related to the growing number of allo-HSCT from HID, older age of donors and recipients, use of new immunosuppressive agents and regimens, increased awareness of the complication, and improved diagnostic tools [9]. EBV PTLD-related mortality was reported to be 84.6% after allo-HSCT before 2000 [10]. Although new approaches for EBV PTLD, including EBV DNA load monitoring and preemptive treatment with rituximab, remarkably improves the outcome of PTLD patients, mortality remains high and approximately one-third of patients die after diagnosis of PTLD [1]. The 3-year survival rate in patients with PTLD is 20% as compared to 62% in those without PTLD [6]. Thus, greater attention of this disorder is warranted.

Here we performed a study in a consecutive series of patients with high-risk hematological malignancies who received unmanipulated allogeneic peripheral blood stem cell transplantation (PBSCT) from either a HID or a MSD in our center and identified the incidence, risk factors, and clinical outcomes of EBV-DNAemia and PTLD. Given that the incidences of EBV-DNAemia and PTLD depend mainly on the degree of HLA matching and, hence, the need for pretransplant T cell depletion protocols, we further made subgroup analyses according to the type of donor.

Methods

Study design

This is a retrospective, observational cohort study enrolled in a total of 200 patients with hematologic malignancies hospitalized at the Chinese PLA General Hospital and underwent HID-SCT or MSD-SCT consecutively between March 2014 and December 2017. Indications included acute leukemia, myelodysplastic syndrome, chronic myeloid leukemia, and lymphoma. Ninety patients received allogeneic PBSCT from a MSD (10 out of 10 HLA markers match) and 110 patients for whom a MSD or HLA-matched unrelated donor was not available received allogeneic PBSCT from a HID (at least 5 out of 10 HLA markers match). The clinical characteristics of patients and donors are described in Table 1. This study was approved by the Ethics Committee of Chinese PLA General Hospital, and signed informed consents were obtained from all patients prior to transplantation in accordance with principles of Declaration of Helsinki.

Conditioning regimen, graft, and GVHD prophylaxis

A total of 173 patients received modified Bu/Cy conditioning regimen consisted of busulfan (Bu, 3.2 mg/kg/day, days − 10 to − 8), carmustine (250 mg, day − 7), cytarabine (4 g/m^2/day, days − 6 to − 5), and cyclophosphamide (Cy, 50 mg/kg/d, days − 4 to − 3). Four patients with refractory acute lymphoblastic leukemia received TBI/Cy conditioning regimen consisted of total body irradiation (8 Gy, day − 7), cytarabine (4 g/m^2/day, days − 6 to − 5), and Cy (60 mg/kg/day, days − 4 to − 3). Eleven patients with organ dysfunction during previous chemotherapy received Flu/Bu conditioning regimen, which was similar to the modified Bu/Cy regimen except that Cy was substituted with fludarabine (Flu, 30 mg/m^2/day, days − 7 to − 3). Twelve patients with relapsed/refractory acute leukemia received Bu/FLAG conditioning regimen consisted of Bu (3.2 mg/kg/day, days − 10 to − 8), Flu (30 mg/m^2/day, days − 7 to − 3), cytarabine (1.6 g/m^2/day, days − 7 to − 3), and granulocyte colony-stimulating factor (G-CSF, 5 μg/kg/day, days − 8 to − 3). ATG (thymoglobulin, rabbit; Genzyme Europe BV; 2.5 mg/kg/day, days − 5 to − 2) was used in all patients of HID-SCT. For recipients of MSD-SCT, ATG (2.5 mg/kg/day, days − 5 to − 4) was used in case of either the donor or the recipient was older than 40 years of age. G-CSF-primed unmanipulated PBSCs were collected and infused into the recipients on the day of collection. None of the patients enrolled in this study had undergone pretransplant splenectomy. The target mononuclear cell count and CD34$^+$ cell count were 5 × 10 [8]/kg and 2 × 10 [6]/kg recipient body weight, respectively. Cyclosporin A, mycophenolate mofetil, and short-term methotrexate were used for GVHD prophylaxis for all recipients as previously reported. For HID-SCT

Table 1 Clinical features of recipients and donors

Variable	Total
No. of patients	200
Age at transplantation, median, years (range)	37 (7–63)
< 40 years	117 (58.5)
≥ 40 years	83 (41.5)
Gender	
Male	128 (64.0)
Female	72 (36.0)
Disease	
Acute myeloid leukemia	103 (51.5)
Myelodysplastic syndromes	23 (11.5)
Chronic myeloid leukemia	6 (3.0)
Myelodysplastic/myeloproliferative neoplasms	1 (0.5)
Acute lymphoblastic leukemia/lymphoma	59 (29.5)
Non-Hodgkin lymphoma	7 (3.5)
Plasma cell leukemia	1 (0.5)
Disease status at PBSCT	
Complete remission	149 (74.5)
Non-remission	51 (25.5)
Pretreatment with ATG	
No ATG	65 (32.5)
ATG	135 (62.5)
Transplantation from HLA-matched sibling donors with ATG	25 (12.5)
Transplantation from HLA-haploidentical related donors with ATG	110 (55.0)
Conditioning regimen	
Non-fludarabine	177 (88.5)
Modified Bu/Cy	173 (86.5)
TBI/Cy	4 (2.0)
Fludarabine-containing	23 (11.5)
Fludarabine/Bu	11 (5.5)
Bu/FLAG	12 (6.0)
Type of donor	
Matched sibling donors	90 (45.0)
Haploidentical related donors	110 (55.0)
Age of donor	
< 40 years	108 (54.0)
≥ 40 years	92 (46.0)
Donor–recipient ABO match	
Match	120 (60.0)
Major mismatch	34 (17.0)
Minor mismatch	36 (18.0)
Bidirectional mismatch	10 (5.0)
Donor–recipient gender match	
Female to male	55 (27.5)
Female to female	27 (13.5)
Male to female	45 (22.5)
Male to male	73 (36.5)
Graft	
Mononuclear cells, median, $\times 10^8$/kg (range)	8.7 (4.8–16.7)
$CD34^+$, median, $\times 10^6$/kg (range)	3.3 (1.1–9.3)

recipients, cyclosporin A was given at 2 mg/kg b.i.d from day − 10 to 3 months after transplantation (trough concentration, 180–250 ng/ml), and then slowly tapered and completely discontinued by 9–12 months in case of no GVHD occurrence. For MSD-SCT recipients, cyclosporin A was given at 2 mg/kg b.i.d from day − 10 to 2 months (trough concentration, 180–250 ng/ml), and then tapered and discontinued at around 6–9 months unless GVHD developed. Mycophenolate mofetil was given at 500 mg b.i.d from day − 10 to 45 days after transplantation for HID-SCT recipients and 30 days after transplantation for MSD-SCT recipients. Following graft infusion, methotrexate was administered intravenously with 15 mg/m^2/day on day + 1 and 10 mg/m^2/day on days + 3, + 6, and + 11 for all recipients.

EBV and CMV monitoring, definitions of viral infection and PTLD, and treatment

Serostatus against EBV or cytomegalovirus (CMV) was detected with immunofluorescence assays for donors and recipients within 1 month before transplantation. All of the donors were EBV IgG positive (+)/IgM negative (−) and CMV IgG+/IgM− in this study. Majority of the recipients were EBV IgG+/IgM− and CMV IgG+/IgM−, except that 1 recipient was EBV IgG−/IgM− and 2 recipients were CMV IgG−/IgM−. EBV DNA and CMV DNA were monitored by real-time quantitative PCR on plasma samples weekly, which was started from the time of granulocyte implantation to + 4 months after transplantation and was then tailored based on continuing immunosuppression, previous history of viral reactivations, and immune reconstitution. The EBV DNAemia or CMV DNAemia was defined as DNA loads ≥ 1000 copies/mL of plasma. The frequency of monitoring should be increased to twice a week in patients with rising DNA copies. The diagnosis of EBV-associated PTLD was defined as proven or probable according to the published definition [11]. Probable PTLD was defined as significant lymphadenopathy, hepatosplenomegaly, or other end-organ manifestations accompanied by significant EBV DNAemia and the absence of other documented cause. Proven PTLD was defined as the detection of EBV in tissue specimen accompanied by symptoms and/or signs from the affected organ [11]. Morphological classification of PTLD was based on histological WHO 2016 classification, which including six types: plasmacytic hyperplasia, infectious mononucleosis-like, florid follicular hyperplasia, polymorphic, monomorphic (B cell or T-/NK-cell types), and classical Hodgkin's lymphoma. All of the HSCT recipients prophylactically received ganciclovir at 10 mg/kg/day from day − 10 to day − 3 followed by acyclovir at 0.8 g/day from day + 1 after transplantation. The preemptive therapy against EBV DNAemia was rituximab at a dose of 375 mg/m^2 once weekly until EBV DNAemia negativity, which was triggered by persistent EBV DNA loads ≥ 10,000 copies/mL accompanied with either persistent fever despite empirical broad-spectrum antibiotic therapy or clinically apparent lymphoproliferative disease. Patients who only showed EBV DNAemia were not given preemptive rituximab. The treatment against EBV PTLD included administration of rituximab, donor lymphocyte infusion, and EBV-specific cytotoxic lymphocyte infusion. Considering the relatively high risks of acute GVHD within 2 months for MSD-SCT or 3 months after transplantation for HID-SCT in the setting of PBSCT, the reduction of immunosuppression would not be considered within this time interval.

Endpoints and definitions

The primary endpoints were the cumulative incidence of post-transplant EBV DNAemia and PTLD. The secondary endpoints included the cumulative incidence of acute GVHD, chronic GVHD, relapse and non-relapse mortality (NRM), overall survival (OS), and relapse-free survival (RFS). Acute GVHD and chronic GVHD were assessed as previously defined [12, 13]. Relapse was defined as the hematologic recurrence of leukemia. NRM was defined as death from any cause without disease relapse. OS was defined as the time from transplantation until death from any cause with censoring of surviving patients at the last follow-up, and RFS was defined as the time from transplantation to relapse or death with censoring of surviving patients at the last follow-up. The time points after transplantation are represented by "+" signs.

Statistical analyses

Clinical features between groups were compared using the 2-sided Fisher's exact test for categorical data and non-parametric Mann–Whitney *U* test for continuous variables. The cumulative incidences of EBV DNAemia, PTLD, GVHD, relapse, and NRM were estimated considering the competing risks. Death without EBV DNAemia was considered as a competing event for EBV DNAemia. Death without PTLD was considered as a competing event for PTLD. For GVHD, relapse and death were considered as competing events. For relapse, transplant-related death was considered as a competing event. For NRM, relapse was considered as a competing event. Univariate analysis for EBV DNAemia, PTLD, GVHD, relapse, and NRM with competing events was performed using Gray's method [14]. The Fine and Gray semi-parametric proportional hazards regression model was used for multivariate analysis to confirm the factors associated with the risks of EBV DNAemia, PTLD, relapse, and NRM [15]. OS and RFS were estimated by the Kaplan–Meier method with the log-rank test for univariate analysis. The Cox proportional hazards regression model with stepwise forward selection was used for multivariate analysis to confirm the factors associated with RFS or OS. Factors for univariate

analysis and multivariate analysis of risk for EBV DNAemia and PTLD were patient's age (< 40-year vs. ≥ 40-year), gender (male vs. female), disease (myeloid malignancies vs. lymphoid malignancies), disease status at HSCT [complete remission (CR) vs. non-remission (NR)], pretreatment with ATG (no vs. yes), conditioning regimen (non-fludarabine vs. fludarabine-containing), type of donor (MSD vs. HID), donor's age (< 40-year vs. ≥ 40-year), donor–recipient ABO match (match vs. mismatch), CMV DNAemia (no vs. yes), and acute GVHD (no vs. yes). Statistical analyses were performed using R statistical software and the cmprsk package (Comprehensive R Archive Network, TU Wien, Austria), Stata 14.0 software (Stata Corporation, College Station, TX, USA), and SPSS 20.0 software (IBM Corporation, Armonk, NY, USA). A p value < 0.05 was chosen as a threshold for significance. The endpoint of follow-up for all surviving subjects was April 30, 2018.

Results

Baseline features of recipients of haploidentical and matched-sibling PBSCT and transplant outcomes

In this study, myeloid malignancies account for approximately 60% of the enrolled patients (Table 1). Of the 67 patients with non-myeloid malignancies, 7 were diagnosed with non-Hodgkin lymphoma. Of them, 2 had extranodal natural killer/T cell lymphoma with EBV DNAemia at initial diagnosis, both of whom did not develop EBV DNAemia and PTLD after transplantation. Another 5 patients with non-Hodgkin lymphoma and all of the patients with acute lymphoblastic leukemia/lymphoma were EBV IgM negative at initial diagnosis. In the 90 recipients of MSD-SCT, 65 did not receive ATG-containing conditioning regimen. A total of 25 recipients of MSD-SCT and all of the 110 recipients of HID-SCT received ATG-containing conditioning regimen. In total recipients, the median time of neutrophil implantation and platelet implantation was 11 (range, 8–32) days and 14 (range, 9–120) days, respectively. In 4 patients (2%), complete donor chimerism was never reached and relapse occurred on day + 30.

Transplant outcomes are shown in Supplementary Table 1. The cumulative incidences of acute GVHD and 2-year chronic GVHD in total cohort were 49.6% and 31.8%, respectively. Similar cumulative incidences of acute GVHD grades 2–4 (28.4% vs. 36.5%, $p = 0.211$) and acute GVHD grades 3–4 (6.1% vs. 5.9%, $p = 0.864$), higher cumulative incidences of chronic GVHD (41.1% vs. 24.4%, $p = 0.016$), and severe chronic GVHD (31.3% vs. 16.7%, $p = 0.018$) occurred in the MSD-SCT group compared with the HID-SCT group. The 2-year cumulative incidences of relapse and NRM in the total cohort were 23.2% and 23.9%, respectively. The estimated 2-year RFS and OS in the total cohort were 51.7% and 58.5%, respectively. Similar 2-year cumulative incidences of relapse (25.8% vs. 23.1%, $p = 0.344$), lower cumulative incidence of 2-year NRM (13.4% vs. 36.3%, $p = 0.004$), and superior 2-year RFS (63.9% vs. 40.9%, $p = 0.002$) and OS (67.3% vs. 46.9%, $p = 0.004$) were recorded in the MSD-SCT group compared with the HID-SCT group.

Incidences of EBV DNAemia and risk factors after haploidentical and matched-sibling PBSCT

The 1-year cumulative incidence of EBV DNAemia was 44.1% (95% CI, 37.1%–50.9%). The median time to EBV DNAemia was 42 days (range, 17–204) after transplantation. In the univariate analysis, 6 factors were significantly associated with the cumulative incidence of EBV reactivation: HLA-haploidentical donors ($p < 0.001$), male donors ($p = 0.002$), CMV DNAemia ($p < 0.001$), acute GVHD ($p = 0.001$), disease in CR status at transplantation ($p = 0.003$), and pretreatment with ATG ($p < 0.001$) (Fig. 1 and Table 2). There was no difference in the cumulative incidence of EBV DNAemia depending on age, gender, primary diagnosis, conditioning regimen, donor's age, and donor–recipient ABO match in the univariate analysis (Table 2). In the multivariate analysis, the significance of pretreatment with ATG ($p = 0.008$), male donors ($p = 0.034$), and CMV DNAemia ($p < 0.001$) was confirmed, and disease in CR status at transplantation was marginally associated with an increased risk of EBV DNAemia ($p = 0.076$) (Table 2).

The 1-year cumulative incidence of EBV DNAemia was 20.0% (95% CI, 7.1%–37.6%) in patients undergoing MSD-SCT pretreated with ATG, which was significantly higher than that in patients not pretreated with ATG [4.8% (95% CI, 1.3%–12.3%), $p = 0.025$, Fig. 2a]. The 1-year cumulative incidence of EBV DNAemia in patients undergoing HID-SCT, all of whom were pretreated with ATG, was 73.7% (95% CI, 63.3%–80.2%), which was significantly higher compared with those patients undergoing MSD-SCT pretreated with ATG ($p < 0.001$). The median time to EBV DNAemia was not significantly different between the HID-SCT group and MSD-SCT with ATG group ($p = 0.230$) or between MSD-SCT with the ATG group and MSD-SCT without the ATG group ($p = 0.571$).

In the MSD-SCT group, 2 factors were significantly associated with the cumulative incidence of EBV reactivation: usage of ATG ($p = 0.025$) and CMV DNAemia ($p < 0.001$) in the univariate analysis (Table 3 and Supplementary Fig. 1). There was no difference in the cumulative incidence of EBV DNAemia depending on age, gender, primary diagnosis, disease status at transplantation, conditioning regimen, donor's gender, donor's age, donor–recipient ABO match, and acute GVHD in the univariate analysis (Table 3). In the multivariate analysis, the significance of

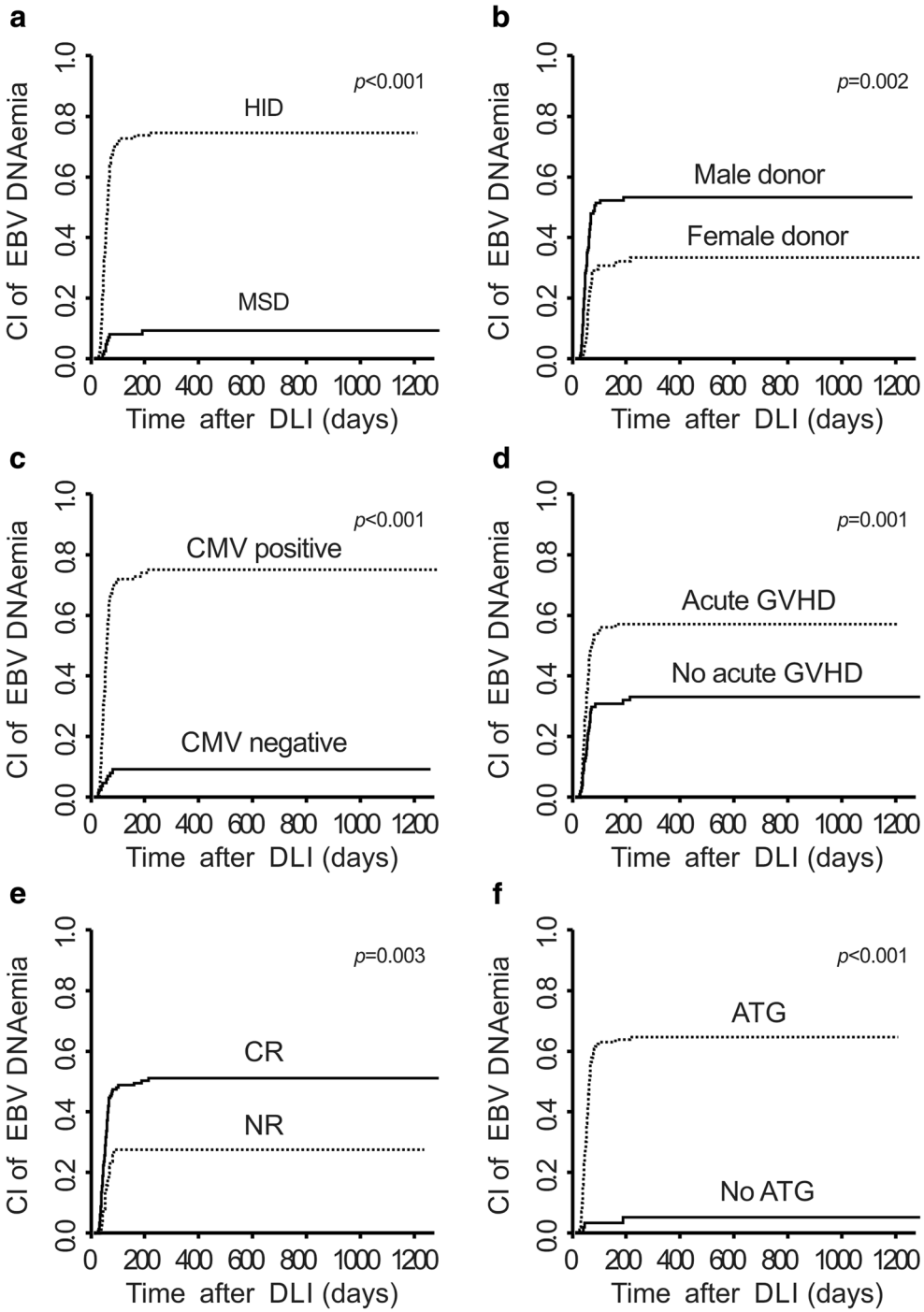

Fig. 1 Comparisons of cumulative incidences of EBV DNAemia according to risk factors in total patients

pretreatment with ATG ($p = 0.006$) and CMV DNAemia ($p = 0.002$) was confirmed, and male donors ($p = 0.073$) and acute GVHD ($p = 0.073$) were marginally associated with an increased risk of EBV DNAemia (Table 3).

In the HID-SCT group, 3 factors were significantly associated with the cumulative incidence of EBV reactivation: donor's age ≥ 40-year ($p = 0.005$), CR status at transplantation ($p = 0.002$), and CMV DNAemia ($p < 0.001$) in the univariate analysis (Table 3 and Supplementary Fig. 2). There was no difference in the cumulative incidence of EBV DNAemia depending on age, gender, primary diagnosis, conditioning regimen, donor's gender, donor–recipient ABO match, and acute GVHD in the univariate analysis (Table 3). In the multivariate analysis, the significance of donor's age ≥ 40 years ($p = 0.028$), CR status at transplantation ($p = 0.022$), and CMV DNAemia ($p = 0.005$) were confirmed (Table 3).

Table 2 Univariate and multivariate analyses for risk factors of EBVemia and PTLD in total cohort

Variable	EBV DNAemia				PTLD			
	Univariate		Multivariate		Univariate		Multivariate	
	% (95% CI)	*p* value	HR (95% CI)	*p* value	% (95% CI)	*p* value	HR (95% CI)	*p* value
Age		0.229				0.115		0.032
< 40 years	48.0 (38.7–56.8)				14.7 (8.4–22.6)		1	
≥ 40 years	38.6 (28.1–48.9)				7.7 (2.8–15.8)		0.4 (0.2–0.9)	
Gender		0.512				0.746		
Male	46.1 (37.3–54.5)				12.6 (7.0–19.9)			
Female	40.4 (28.9–51.5)				10.5 (4.2–20.2)			
Disease		0.526				0.509		
Myeloid malignancies	43.0 (34.4–51.2)				13.2 (7.6–20.5)			
Lymphoid malignancies	46.5 (34.1–58.0)				9.0 (3.2–18.5)			
Disease status at PBSCT		0.003		0.076		0.413		
Complete remission	49.9 (41.5–57.7)		1		13.0 (7.6–19.9)			
Non-remission	27.5 (16.0–40.2)		0.6 (0.4–1.1)		9.0 (2.8–19.7)			
Pretreatment with ATG		< 0.001		0.008		0.001		0.350
No ATG	4.8 (1.3–12.3)		1		1.8 (0.1–8.4)		1	
ATG	63.0 (54.2–70.6)		6.3 (1.6–24.0)		18.6 (11.5–27.1)		2.9 (0.3–27.5)	
Conditioning regimen		0.713				0.022		0.010
Non-fludarabine	44.8 (37.3–52.0)				9.1 (5.1–14.6)		1	
Fludarabine-containing	39.1 (19.3–58.5)				27.4 (10.7–47.2)		3.8 (1.4–10.6)	
Donor's type		< 0.001		0.130		< 0.001		0.350
Matched sibling donors	9.0 (4.2–16.1)		1		2.4 (0.5–7.7)		1	
Haploidentical donors	72.8 (63.3–80.2)		2.0 (0.8–5.1)		23.5 (14.2–34.2)		2.0 (0.5–8.3)	
Donor's gender		0.002		0.034		0.201		0.870
Male	52.7 (43.2–61.3)		1		14.6 (8.1–22.8)		1	
Female	31.8 (22.0–42.0)		0.6 (0.4–1.0)		8.5 (3.4–16.5)		0.9 (0.4–2.4)	
Donor's age		0.510				0.792		
< 40 years	14.2 (5.7–26.5)				11.5 (5.8–19.3)			
≥ 40 years	4.3 (35.2–55.5)				12.2 (5.9–20.9)			
Donor–recipient ABO match		0.513				0.852		
Match	42.6 (33.6–51.3)				12.3 (6.7–19.7)			
Mismatch	46.3 (35.0–56.9)				11.1 (4.8–20.4)			
CMV DNAemia		< 0.001		< 0.001		< 0.001		0.036
No	8.9 (4.1–15.9)		1		1.2 (0.1–5.6)		1	
Yes	73.1 (63.6–80.6)		5.9 (2.5–13.9)		25.4 (15.6–36.3)		11.6 (1.2–114.4)	
Acute GVHD		0.001		0.960		0.134		0.480
No	31.8 (22.9–41.0)		1		8.7 (3.8–16.2)		1	
Yes	56.7 (46.3–65.9)		1.0 (0.7–1.6)		15.2 (8.3–24.1)		1.4 (0.5–3.8)	

CI confidence interval, *GVHD* graft-versus-host disease, *PBSCT* peripheral blood stem cell transplantation

Incidences of PTLD and risk factors after haploidentical and matched-sibling PBSCT

A total of 19 patients developed EBV PTLD at a median of 55 days (range, 36–178) after transplantation (Supplementary Table 2), all of whom were accompanied by EBV DNAemia. Of them, 4 proven PTLD cases were diagnosed from significant lymphadenopathy together with the detection of EBV-encoded RNA by in situ hybridization in biopsy tissues from lymph nodes. Fifteen probable PTLD cases were diagnosed from persistent high fever and significant lymphadenopathy together with high-load EBV DNAemia (≥ 10,000 copies/mL). The EBV-associated hemophagocytic lymphohistiocytosis after transplantation could be ruled out

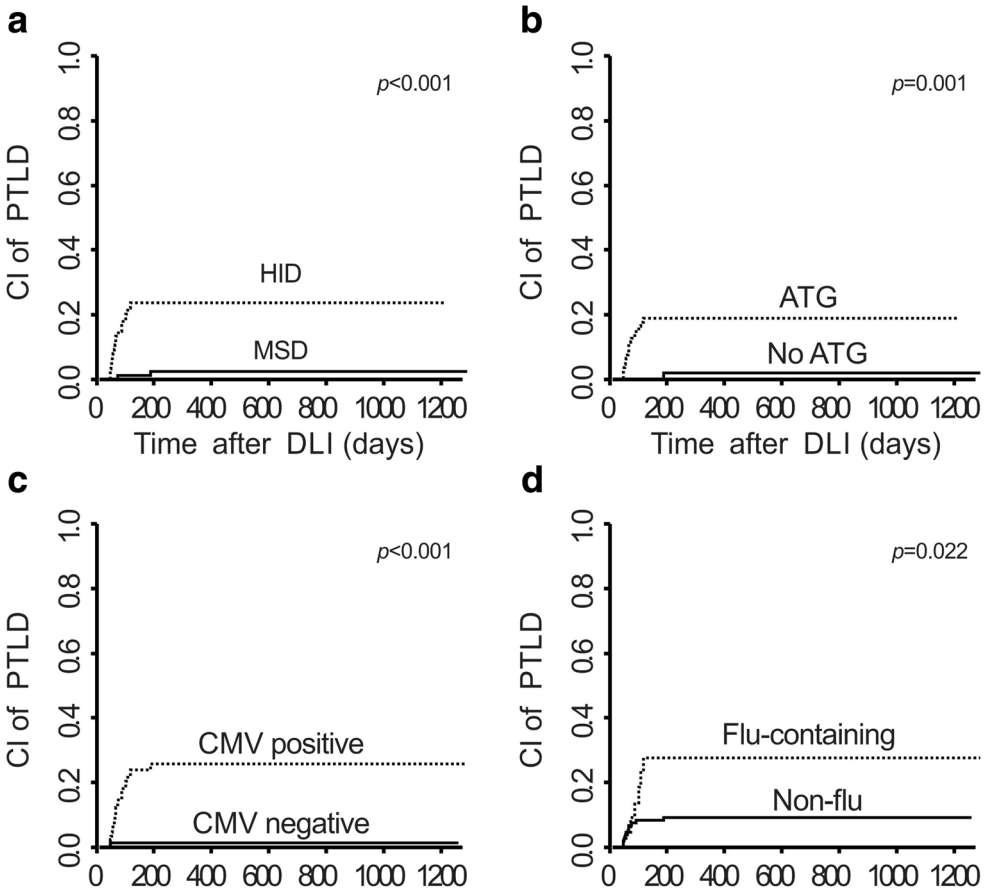

Fig. 2 Comparisons of cumulative incidences of EBV PTLD according to risk factors in total patients

in these 15 patients because typical clinical and laboratory manifestations such as progressive cytopenia, splenomegaly, and significant change from pretransplant baseline in levels of ferritin, triglyceride, or fibrinogen did not occur throughout the course of the disease. All patients with PTLD were administrated with rituximab. Of them, 2 were combined with EBV-specific cytotoxic lymphocyte and 1 was combined with donor lymphocyte infusion. None of them received reduction of immunosuppression. One-year cumulative incidences of total, probable, and proven PTLD were 11.9% (95% CI, 7.4%–11.4%), 9.3% (95% CI, 5.4%–14.4%), and 2.6% (95% CI, 0.9%–6.1%), respectively. In patients with EBV DNAemia, 1-year cumulative incidence of total PTLD was 53.1% (95% CI, 31.4%–70.8%). In the univariate analysis, there was no difference in the cumulative incidence of PTLD depending on gender, primary diagnosis, disease status at transplantation, donor's age, donor's gender, and donor–recipient ABO match (Table 2). Four risk factors were significantly associated with the development of PTLD: HLA-haploidentical donors ($p < 0.001$), pretreatment with ATG ($p = 0.001$), CMV DNAemia ($p < 0.001$), and fludarabine-containing conditioning regimen ($p = 0.022$) (Fig. 2 and Table 2). In the multivariate analysis, the significance of fludarabine-containing conditioning regimen ($p = 0.010$) and CMV DNAemia ($p = 0.036$) was confirmed, and patient's age < 40 years ($p = 0.032$) was also associated with an increased risk of PTLD (Table 2).

Only 2 of 90 (2.2%) patients undergoing MSD-SCT were recorded symptoms of PTLD, while 17 of 110 (15.5%) patients undergoing HID-SCT developed PTLD-related symptoms. One-year cumulative incidence of PTLD was 1.8% (95% CI, 0.1%–8.4%) in MSD-SCT recipients not pretreated with ATG, 4.4% (95% CI, 0.3–18.8%) in MSD-SCT recipients pretreated with ATG, and 23.5% (95% CI, 14.2–34.2%) in HID-SCT recipients. Because of the small number of patients with PTLD in the MSD-SCT group, univariate and multivariate analyses of risk factors for PTLD were not conducted in the MSD-SCT group. In the HID-SCT group, there was a trend toward a higher incidence of PTLD rate in patients with age ≥ 40 years ($p = 0.057$) or those who have CMV DNAemia ($p = 0.057$) in the univariate analysis (Table 4 and Supplementary Fig. 3). There was no significant difference in the cumulative incidence of PTLD depending on age, gender, primary diagnosis, disease status at transplantation, conditioning regimen, donor's gender, donor–recipient ABO match, and acute GVHD in the univariate analysis (Table 4). In the multivariate analysis, fludarabine-containing conditioning regimen was marginally associated with an increased risk of PTLD ($p = 0.057$; Table 4).

Table 3 Univariate and multivariate analyses for risk factors of EBV DNAemia after HLA haploidentical and matched-sibling PBSCT

Variable	MSD-SCT				HID-SCT			
	Univariate		Multivariate		Univariate		Multivariate	
	% (95% CI)	*p* value	HR (95% CI)	*p* value	% (95% CI)	*p* value	HR (95% CI)	*p* value
Age		0.925				0.834		
< 40 years	9.0 (2.8–19.7)				73.2 (61.1–82.1)			
≥ 40 years	9.0 (2.9–19.9)				74.4 (56.9–85.6)			
Gender		0.397				0.591		
Male	11.2 (4.5–21.2)				73.1 (61.2–81.9)			
Female	5.6 (1.0–16.5)				75.0 (56.7–86.4)			
Disease		0.516				0.409		
Myeloid malignancies	7.8 (2.9–16.2)				76.5 (64.2–85.0)			
Lymphoid malignancies	12.0 (2.9–28.0)				69.6 (52.5–81.6)			
Disease status at PBSCT		0.704				0.002		0.028
Complete remission	8.3 (3.0–17.1)				80.6 (70.4–87.6)		1	
Non-remission	10.7 (2.6–25.4)				47.8 (26.2–66.7)		0.5 (0.3–0.9)	
Pretreatment with ATG		0.025		0.006				
No ATG	4.8 (1.3–12.3)		1		–			
ATG	20.0 (7.1–37.6)		6.9 (1.8–26.9)		–			
Conditioning regimen		0.235				0.258		
Non-fludarabine	7.8 (3.1–15.1)				74.5 (64.5–82.1)			
Fludarabine-containing	18.2 (2.4–45.7)				66.7 (29.3–87.5)			
Donor's gender		0.109		0.073		0.186		0.170
Male	14.2 (5.7–26.5)		1		74.7 (63.0–83.1)		1	
Female	4.3 (0.8–12.9)		0.3 (0.1–1.1)		71.4 (52.7–83.8)		0.7 (0.5–1.1)	
Donor's age		0.220				0.005		0.022
< 40 years	5.2 (0.9–15.7)				67.3 (54.4–77.3)		1	
≥ 40 years	12.2 (4.9–23.1)				83.7 (68.1–92.1)		1.7 (1.1–2.6)	
Donor–recipient ABO match		0.635				0.515		
Match	10.0 (4.0–19.2)				76.3 (62.9–85.4)			
Mismatch	6.9 (1.2–20.0)				70.6 (55.6–81.3)			
CMV DNAemia		< 0.001		0.002		< 0.001		0.005
No	2.9 (0.5–9.1)		1		28.6 (11.2–48.8)		1	
Yes	29.7 (11.5–50.7)		10.4 (2.4–45.6)		84.5 (74.8–90.7)		3.9 (1.5–10.2)	
Acute GVHD		0.102		0.073		0.320		
No	5.5 (1.4–13.8)		1		65.9 (49.5–78.1)			
Yes	15.2 (5.4–29.5)		3.1 (0.9–10.4)		77.3 (64.9–85.8)			

CI confidence interval, *GVHD* graft-versus-host disease, *HID* haploidentical donors, *MSD* matched-sibling donors, *PBSCT* peripheral blood stem cell transplantation

Association of EBV DNAemia with clinical outcomes

The median follow-up time of all the surviving allogenic PBSCT recipients was 502 days (range, 67–1286). For the total cohort, the estimated 2-year RFS rate was 56.3% (95% CI, 45.5–65.7%) for patients without EBV DNAemia and 48.7% (95% CI, 35.4–60.7%) for those with EBV DNAemia, respectively, which was not significantly different from each other ($p = 0.150$) (Fig. 3a). The estimated 2-year OS rate in patients without EBV DNAemia was 62.9% (95% CI, 52.2–71.9%), which was not significantly different than those with EBV DNAemia [54.9% (95% CI, 41.4–66.6%); $p = 0.158$] (Fig. 3b). The estimated 2-year relapse rate in patients without EBV DNAemia was 28.6% (95% CI, 19.8–38.1%), which was not significantly different than those with EBV DNAemia [16.8% (95% CI, 9.2–26.3%); $p = 0.268$] (Fig. 3c). The estimated 2-year NRM rate in patients without EBV DNAemia was 15.3% (95% CI, 8.9–23.3%), which was

Table 4 Univariate and multivariate analyses for risk factors of EBV PTLD in HID-SCT recipients

Variable	Univariate		Multivariate	
	% (95% CI)	*p* value	HR (95% CI)	*p* value
Age		0.423		
< 40 years	23.4 (12.5–36.1)			
≥ 40 years	21.5 (7.5–40.1)			
Gender		0.666		
Male	22.2 (11.6–35.0)			
Female	26.6 (9.9–46.9)			
Disease		0.252		
Myeloid malignancies	29.4 (15.8–44.4)			
Lymphoid malignancies	15.6 (5.4–30.7)			
Disease status at PBSCT		0.335		
Complete remission	25.5 (14.5–38.0)			
Non-remission	17.5 (3.9–39.1)			
Conditioning regimen		0.116		0.057
Non-fludarabine	17.9 (9.6–28.3)		1	
Fludarabine-containing	45.5 (14.3–72.7)		2.3 (1.0–5.4)	
Donor's gender		0.546		
Male	24.5 (13.2–37.6)			
Female	22.1 (7.4–41.5)			
Donor's age		0.057		0.170
< 40 years	16.9 (7.7–29.2)		1	
≥ 40 years	37.5 (16.7–58.4)		1.9 (0.8–4.8)	
Donor–recipient ABO match		0.641		
Match	25.8 (12.8–40.9)			
Mismatch	21.0 (8.8–36.6)			
CMV DNAemia		0.057		0.150
No	5.6 (0.3–23.1)		1	
Yes	30.3(18.0–43.5)		4.9 (0.6–43.7)	
Acute GVHD		0.685		
No	23.4 (9.0–41.7)			
Yes	23.6(12.2–37.1)			

CI confidence interval, *GVHD* graft-versus-host disease, *PBSCT* peripheral blood stem cell transplantation

significantly lower than those with EBV DNAemia [35.8% (95% CI, 23.8–48.0%); $p = 0.002$] (Fig. 3d).

In the patients undergoing MSD-SCT, the estimated 2-year RFS rate was 64.0% (95% CI, 51.5–74.1%) for those without EBV DNAemia and 57.1% (95% CI, 17.2–83.7%) for those with EBV DNAemia, respectively, which was not significantly different ($p = 0.707$) (Supplementary Fig. 4A). The estimated 2-year OS rate in patients without EBV DNAemia was 70.8% (95% CI, 58.6–80.0%), which was not significantly different than those with EBV DNAemia [57.1% (95% CI, 17.2–83.7%); $p = 0.496$] (Supplementary Fig. 4B). The estimated 2-year relapse rate in patients without EBV DNAemia was 24.2% (95% CI, 14.7–34.9%), which was not significantly different than those with EBV DNAemia [14.3% (95% CI, 0.5–49.6%); $p = 0.649$] (Supplementary Fig. 4C). The estimated 2-year NRM rate in patients without EBV DNAemia was 11.9% (95% CI, 5.8–20.4%), which was not significantly different than those with EBV DNAemia [28.6% (95% CI, 3.0–63.9%); $p = 0.257$] (Supplementary Fig. 4D).

In the patients undergoing HID-SCT, the estimated 2-year RFS rate was 34.8% (95% CI, 16.1–54.3%) for those without EBV DNAemia and 49.9% (95% CI, 36.9–61.7%) for those with EBV DNAemia, respectively, which was not significantly different from each other ($p = 0.296$) (Supplementary Fig. 5A). The estimated 2-year OS rate in patients without EBV DNAemia was 39.5% (95% CI, 19.4–59.1%), which was not significantly different than those with EBV DNAemia [57.6% (95% CI, 44.7–68.6%); $p = 0.371$] (Supplementary Fig. 5B). The estimated 2-year relapse rate in patients without EBV DNAemia was 39.8% (95% CI,

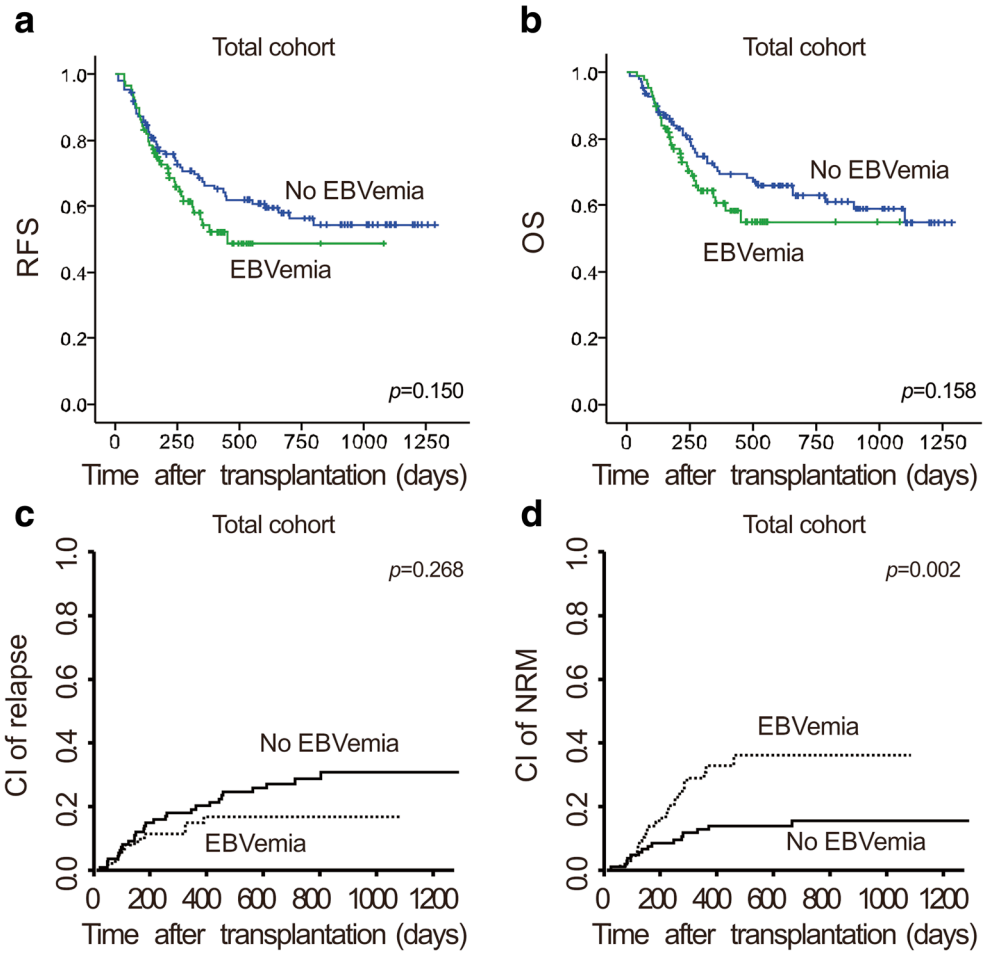

Fig. 3 Comparisons of transplant outcomes according to EBV serostatus in total patients

21.2–57.9%), which was significantly higher than those with EBV DNAemia [16.8% (95% CI, 8.9–26.8%); $p = 0.013$] (Supplementary Fig. 5C). The estimated 2-year NRM rate in patients without EBV DNAemia was 26.0% (95% CI, 9.2–46.7%), which was not significantly different than those with EBV DNAemia [34.7% (95% CI, 23.2–46.5%); $p = 0.185$] (Supplementary Fig. 5D).

In the multivariate analysis, EBV DNAemia was associated with a lower risk of relapse ($p = 0.025$) and was not an independent risk factor for RFS, OS and NRM (Supplementary Table 3).

Association of PTLD with clinical outcomes

Eleven of the 19 (57.9%) patients with PTLD died, all of whom received HID-SCT, and the median time to death was 93 days (range, 5–355) after the occurrence of PTLD (Supplementary Table 2). PTLD was the primary or contributing case of death in 3 patients, while 5 patients died of relapse, 1 patient died of GVHD, and 5 patients died of pneumonia. Of the 5 patients who died of relapse after the onset of PTLD, 1 (patient no. 3) was diagnosed as proven PTLD with typical pathological findings and 4 (patient no. 5, no. 6, no. 7, and no. 9) were diagnosed as probable PTLD with high EBV DNA loads accompanied with persistent fever and lymphadenopathy. Among the 4 probable PTLD cases, patient no. 6, no. 7, and no. 9 were diagnosed as acute myeloid leukemia and patient no. 5 was diagnosed as T-lymphoblastic leukemia/lymphoma. Patient no. 5 was diagnosed as probable PTLD on day + 53 after transplantation but not a relapse because the bone marrow pathology and chimera examination at + 2 months, + 3 months, and + 4 months after transplantation did not suggest any evidence of leukemia relapse and his signs and syndromes of fever and multiple lymphadenopathy were relieved after treatment with preemptive rituximab. None of the 11 surviving patients with probable PTLD developed relapse; therefore, the possibility of relapse at the onset of PTLD in these patients could be ruled out. In the total cohort, the estimated 1-year RFS rate was 63.4% (95% CI, 55.4–70.4%) for patients without PTLD, which was significantly higher than those with PTLD [40.2% (95% CI, 18.4–61.2%); $p = 0.013$] (Fig. 4a). The estimated 1-year OS rate in patients without PTLD was 68.4% (95% CI, 60.4–75.0%), which was significantly higher than those with PTLD [44.9% (95% CI, 21.9–65.6%); $p = 0.012$] (Fig. 4b). The estimated 1-year relapse rate in patients without PTLD was lower than in those with PTLD with

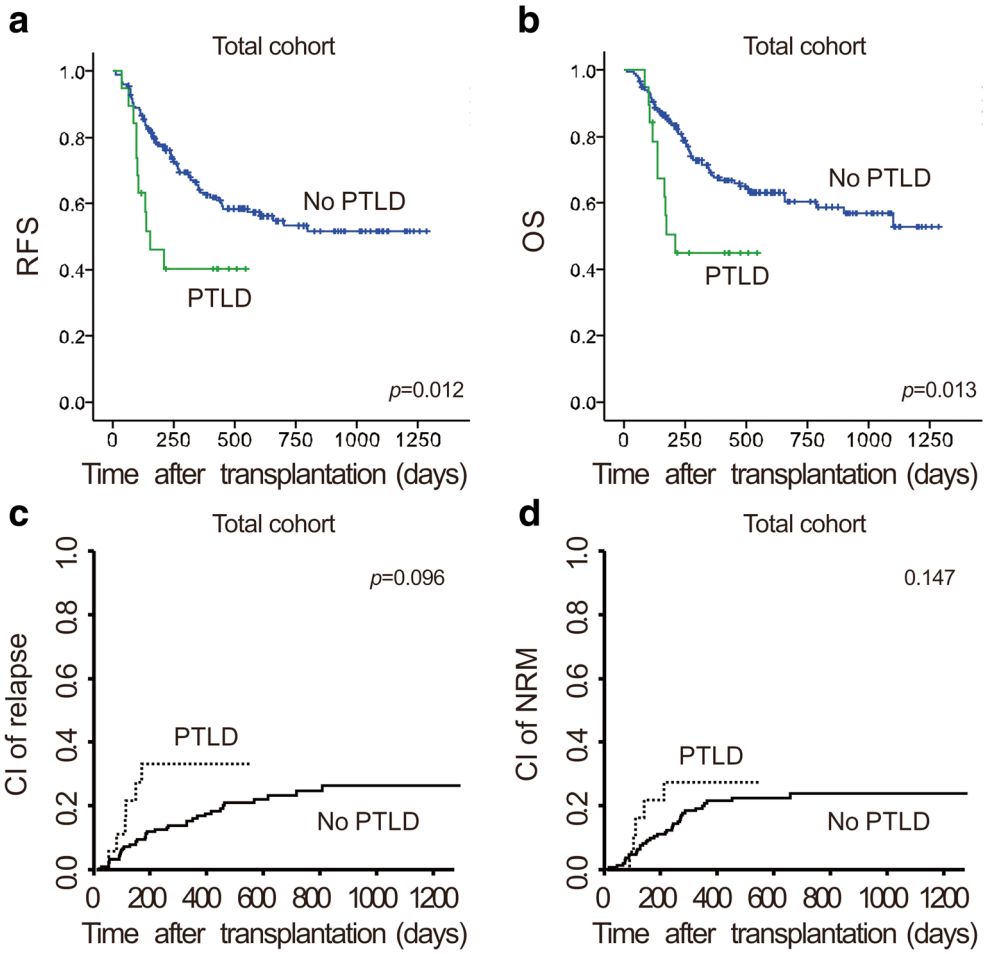

Fig. 4 Comparisons of transplant outcomes according to the occurrence of EBV PTLD in total patients

marginal significance [16.2% (95% CI, 11.1–22.3%) vs. 32.5% (95% CI, 12.6–54.4%); $p = 0.096$] (Fig. 4c). The estimated 1-year NRM rate in patients without PTLD was 21.1% (95% CI, 15.1–27.8%), which was not significantly different than those with PTLD [27.0% (95% CI, 9.3–48.6%); $p = 0.385$] (Fig. 4d).

In the patients undergoing HID-SCT, the estimated 1-year RFS rate was 51.5% (95% CI, 39.4–62.4%) for those without PTLD, which was significantly higher than those with PTLD [35.3% (95% CI, 14.5–57.0%); $p = 0.046$] (Supplementary Fig. 6A). The estimated 1-year OS rate in patients without PTLD was 51.5% (95% CI, 39.4–62.4%), which was significantly higher than that in those with PTLD [35.3% (95% CI, 14.5%–57.0%); $p = 0.044$] (Supplementary Fig. 6B). The estimated 1-year relapse rate in patients without PTLD was 19.1% (95% CI, 11.3–28.5%), which was significantly lower than that in those with PTLD [35.3% (95% CI, 13.7–58.9%); $p = 0.046$] (Supplementary Fig. 6C). The estimated 1-year NRM rate in patients without PTLD was 30.6% (95% CI, 20.4–41.4%), which was not significantly different than those with PTLD [29.4% (95% CI, 10.1–52.1%); $p = 0.124$] (Supplementary Fig. 6D).

In the multivariate analyses, PTLD was marginally associated with an increased risk of relapse ($p = 0.092$) and was not an independent risk factor for RFS, OS, and NRM (Supplementary Table 3).

Discussion

In the current study, we assessed the incidences and clinical features of EBV DNAemia and PTLD in 200 consecutive PBSCT recipients from either an HLA-haploidentical donor or a matched-sibling donor. Our results showed that the overall incidence of EBV DNAemia was 44.1%, ranging from 4.8% in MSD-SCT recipients not pretreated with ATG to 20.0% in MSD-SCT recipients pretreated with ATG, and 73.7% in HID-SCT recipients. Several independent risk factors were associated with reactivation of EBV after transplantation, including use of ATG, male donor, and reactivation of CMV. The overall incidence of EBV PTLD was 11.9%, ranging from 1.8% in MSD-SCT recipients not pretreated with ATG to 4.4% in MSD-SCT recipients pretreated with ATG, and 23.5% in HID-SCT recipients. EBV PTLD was frequent

in the EBV DNAemia-positive subgroup, with an overall incidence of 21.3%. An independent risk factor associated with EBV PTLD after HID-SCT was fludarabine-containing conditioning regimen. According to the univariate analysis, neither RFS nor OS was inferior in recipients with EBV DNAemia compared with recipients without EBV DNAemia. The NRM rate in patients with EBV DNAemia was significantly higher than that in those without EBV DNAemia (35.8% vs. 15.3%), but this difference was not significant anymore in the subgroup analysis according to the donor's type. Although the risk of relapse was not different in patients with EBV DNAemia compared with those without EBV DNAemia in the total cohort, in the HID-SCT subgroup the risk of relapse in patients with EBV DNAemia was significantly lower than those without EBV DNAemia (16.8% vs. 39.8%). Remarkably, both RFS and OS were inferior in patients with EBV PTLD compared with those without EBV PTLD (40.2% vs. 63.4% and 44.9% vs. 68.4%, respectively). In the patients undergoing HID-SCT, the relapse rate was higher in those with PTLD than in those without PTLD (35.3% vs. 19.1%). The NRM rate in patients with PTLD was not higher than in those without PTLD. According to the multivariate analysis, neither EBV DNAemia nor PTLD independently predicted inferior RFS, OS, and NRM. EBV DNAemia predicted a lower risk of relapse (HR 0.3), while PTLD weakly predicted a higher risk of relapse (HR 3.0).

There is no consensus on the specimen type for EBV viral load monitoring; thus, the overall incidence of EBV DNAemia and the cut-off level of EBV-DNA chosen to start a preemptive therapy vary widely in different studies [1–5]. Incidence of post-transplant EBV reactivation has been observed to vary considerably in different studies. The overall frequency of EBV reactivation in this study was 44.1%. Therefore, a high incidence was observed because 62.5% of the study patients received ATG containing conditioning regimen. This was consistent with the suggestion of previous studies that there might be a strong association of EBV reactivation with the use of ATG [1]. Also, the frequency of EBV reactivation in the subgroup of patients who received ATG in this study was 63%, which was similar with the report of Cesaro et al. [5] that all patients received a conditioning regimen containing ATG. These data support the well-known mechanism that action of ATG is mainly dependent on in vivo T cell depletion. Searching for associations between EBV reactivation and other clinical risk factors, transplant recipients of female donor had a 40% lower risk of EBV reactivation when compared to recipients of male donor in this study. To our knowledge, the association between donor's gender and EBV reactivation has not been reported preciously; therefore, study is needed to replicate this finding and further clarify the underlying mechanism. Our results confirmed that CMV DNAemia was strongly associated with a 5.9-fold higher risk of EBV DNAemia, which was consistent with the report of Zallio et al. [16] that reactivation of CMV was an independent variable associated with reactivation of EBV. This finding suggests the potential important role of CMV in predicting reactivation of EBV. Either conditioning regimen or intensification of immunosuppressive therapy (high-dose steroids) for GVHD can induce post-transplant CMV reactivation by suppressing anti-CMV T cell–specific function or by directly activating viral replication. Therefore, reactivation of CMV itself reflects the severity of immunosuppression [17]. Further, in vitro studies support the suggestion that a CMV infection is related to an EBV reactivation and CMV might be important as a co-factor in EBV reactivation and pathogenesis in immunocompromised patients [18]. In the subgroup analysis according to the donor's type in this study, the association between EBV reactivation and use of ATG or CMV DNAemia was confirmed in MSD-SCT, as suggested in the total cohort analysis. It was worth to be notified that in HID-SCT recipients, all of whom received ATG treatment, donor's age ≥ 40 years and CR status at transplantation were associated with a higher risk of EBV replication. A study has shown a correlation between the number of viruses and the donors' age, consistent with a higher likelihood for virus exposure in older donors [18]. Further, distinct viral infections might play a role as a potential cofactor in promoting EBV replication in immunocompromised patients [16]. In this study, we showed that CR status at transplantation in the HID-SCT group was associated with an increased cumulative incidence of EBV reactivation, as compared with NR status at transplantation (80.6% vs. 47.8%). This evidence provided an explanation for the association between EBV reactivation and a lower risk of relapse among the HID-SCT recipients. Further, it has been reported that prompt B cell reconstitution favors EBV reactivation after in HSCT recipients because of almost all cases arise from donor-derived EBV-infected B cells [19]. Relapse in patients following allo-HSCT showed an association with decreased numbers of B cells [20]. Based on these findings, we hypothesized that pretransplant NR status of disease favored relapse after HID-SCT and delayed donor B cell reconstitution and thereby decreased EBV DNA load. To our knowledge, the inverse correlation between EBV and relapse in HID-SCT setting has not been reported preciously; therefore, study is needed to replicate these findings and further clarify the underlying mechanism.

Almost all of EBV PTLDs are of donor origin and develop during 2 to 4 months following allo-HSCT, due to profound T cell depleting conditioning regimen leading to impaired anti-EBV cellular immunity and hence quick growth of EBV-positive B cells. The most common symptoms and signs of EBV PTLD are fever and lymphadenopathy, which may rapidly progress to multiorgan failure and even death [21, 22]. A surprising finding in the current study was a high incidence of PTLD of 23.5% in HID-SCT recipients. A total of 17 patients developed PTLD in the HID-SCT recipients, including 3 histologically

confirmed cases. It has been reported that the incidence of PTLD after allo-HSCT was between 1.2 and 11.2% depending on the HSCT settings [1]. In contrast to our result, previous study by Xu et al. has shown that 1 year cumulative incidence of PTLD after HID-SCT using unmanipulated bone marrow cells plus PBSCs as graft was 3.0% [23]. It is worth noting that the conditioning regimen in the study of Xu et al. was Bu/Cy, not containing fludarabine. However, in the current study 11.5% of patients received fludarabine-containing conditioning regimens and use of fludarabine-containing regimens conferred a 2.3-fold increased risk for EBV PTLD. Fludarabine has been shown to be associated with the development of EBV PTLD [24, 25]. We therefore hypothesized that the higher incidence of PTLD in our study compared to previous studies [1, 23] was a consequence of using fludarabine-containing conditioning regimens. Certainly, it is important to note that approximately 75% of patients diagnosed with PTLD in this study lack pathological diagnosis. Therefore, the finding of the unexpected high incidence of PTLD in HID-SCT needs to be confirmed in a larger trial. In previous studies, younger age and EBV serology donor/recipient mismatch have been suggested to be associated with increased risk of PTLD [1, 26]. In the current study, CMV DNAemia and patient's age < 40 years were independently associated with increased risks of PTLD in total HSCT recipients; however, they were not statistically significant anymore in the HID-SCT recipients in the multivariate analysis model. Because in the current study nearly all of the donors and recipients were EBV IgG+/IgM− except one EBV IgG−/IgM− recipient, we therefore could not further identify the influence of serological EBV mismatch on PTLD after transplantation.

In this study, EBV PTLD was associated with inferior RFS and OS in univariate analysis, which was in accordance with the results of other research [24]. But EBV PTLD was not an independent risk factor for survival in multivariate analysis, suggesting that other factors might have a potential interaction with PTLD. It is clear from our result that even if PTLD leads to death in some cases, majority of the patients with PTLD eventually succumb from other reasons including relapse and pneumonia. The presence of PTLD might mirror a poor immune reconstitution, and meanwhile, treatment of PTLD further aggravated the suppression of immunity. This hypothesis requires further verification.

In summary, the data from this study confirmed higher incidences of EBV DNAemia and PTLD after HID-SCT than MSD-SCT in the setting of unmanipulated with PBSCs as graft. Risk factors associated with reactivation of EBV were included in use of ATG, male donor, and CMV DNAemia. EBV PTLD developed mainly in HID-SCT recipients with EBV DNAemia. Use of conditioning regimen containing fludarabine was a risk factor for PTLD after HID-SCT. Although patients with EBV DNAemia did not have an inferior survival, they did have an increased risk of developing EBV PTLD, which was associated with worse RFS and OS after HID-SCT.

Funding This work was partially supported by grants from the National Natural Science Foundation of China (81770203 to D-H L, 81670135 and 81870109 to X-N G), Natural Science Foundation of Beijing (7162174 to J L) and National Clinical Specialist Focus on Military Construction Projects.

Compliance with ethical standards

This study was approved by the Ethics Committee of Chinese PLA General Hospital, and signed informed consents were obtained from all patients prior to transplantation in accordance with principles of Declaration of Helsinki.

Conflict of interest The authors declare no competing interests.

References

1. Styczynski J, Gil L, Tridello G, Ljungman P, Donnelly JP, van der Velden W, Omar H, Martino R, Halkes C, Faraci M, Theunissen K, Kalwak K, Hubacek P, Sica S, Nozzoli C, Fagioli F, Matthes S, Diaz MA, Migliavacca M, Balduzzi A, Tomaszewska A, Camara Rde L, van Biezen A, Hoek J, Iacobelli S, Einsele H, Cesaro S Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation (2013) Response to rituximab-based therapy and risk factor analysis in Epstein Barr Virus-related lymphoproliferative disorder after hematopoietic stem cell transplant in children and adults: a study from the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. Clin Infect Dis 57(6):794–802
2. Ocheni S, Kroeger N, Zabelina T, Sobottka I, Ayuk F, Wolschke C, Muth A, Lellek H, Petersen L, Erttmann R, Kabisch H, Zander AR, Bacher U (2008) EBV reactivation and post transplant lymphoproliferative disorders following allogeneic SCT. Bone Marrow Transplant 42(3):181–186
3. Worth A, Conyers R, Cohen J, Jagani M, Chiesa R, Rao K, Goulden N, Veys P, Amrolia PJ (2011) Pre-emptive rituximab based on viraemia and T cell reconstitution: a highly effective strategy for the prevention of Epstein-Barr virus-associated lymphoproliferative disease following stem cell transplantation. Br J Haematol 155(3):377–385
4. García-Cadenas I, Castillo N, Martino R, Barba P, Esquirol A, Novelli S, Orti G, Garrido A, Saavedra S, Moreno C, Granell M, Briones J, Brunet S, Navarro F, Ruiz I, Rabella N, Valcárcel D, Sierra J (2015) Impact of Epstein Barr virus-related complications after high-risk allo-SCT in the era of pre-emptive rituximab. Bone Marrow Transplant 50(4):579–584
5. Cesaro S, Pegoraro A, Tridello G, Calore E, Pillon M, Varotto S, Abate D, Barzon L, Mengoli C, Carli M, Messina C (2010) A prospective study on modulation of immunosuppression for Epstein-Barr virus reactivation in pediatric patients who underwent unrelated hematopoietic stem-cell transplantation. Transplantation 89(12):1533–1540
6. Uhlin M, Wikell H, Sundin M et al (2014) Risk factors for Epstein-Barr virus-related post-transplant lymphoproliferative disease after allogeneic hematopoietic stem cell transplantation. Haematologica. 99(2):346–352
7. Landgren O, Gilbert ES, Rizzo JD, Socié G, Banks PM, Sobocinski KA, Horowitz MM, Jaffe ES, Kingma DW, Travis LB, Flowers ME, Martin PJ, Deeg HJ, Curtis RE (2009) Risk factors for lymphoproliferative disorders after allogeneic hematopoietic cell transplantation. Blood 113(20):4992–5001
8. Dierickx D, Habermann TM (2018) Post-transplantation lymphoproliferative disorders in adults. N Engl J Med 378(6):549–562

9. Bollard CM, Heslop HE (2016) T cells for viral infections after allogeneic hematopoietic stem cell transplant. Blood 127(26): 3331–3340
10. Curtis RE, Travis LB, Rowlings PA, Socié G, Kingma DW, Banks PM, Jaffe ES, Sale GE, Horowitz MM, Witherspoon RP, Shriner DA, Weisdorf DJ, Kolb HJ, Sullivan KM, Sobocinski KA, Gale RP, Hoover RN, Fraumeni JF Jr, Deeg HJ (1999) Risk of lymphoproliferative disorders after bone marrow transplantation: a multi-institutional study. Blood 94(7):2208–2216
11. Styczynski J, Reusser P, Einsele H, de la Camara R, Cordonnier C, Ward KN, Ljungman P, Engelhard D; Second European Conference on Infections in Leukemia (2009) Management of HSV, VZV and EBV infections in patients with hematological malignancies and after SCT: guidelines from the Second European Conference on Infections in Leukemia. Bone Marrow Transplant 43(10):757–770
12. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, Thomas ED (1995) 1994 Consensus conference on acute GVHD grading. Bone Marrow Transplant 15(6):825–828
13. Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW, Palmer J, Weisdorf D, Treister NS, Cheng GS, Kerr H, Stratton P, Duarte RF, McDonald GB, Inamoto Y, Vigorito A, Arai S, Datiles MB, Jacobsohn D, Heller T, Kitko CL, Mitchell SA, Martin PJ, Shulman H, Wu RS, Cutler CS, Vogelsang GB, Lee SJ, Pavletic SZ, Flowers ME (2015) National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. the 2014 Diagnosis and Staging Working Group report. Biol Blood Marrow Transplant 21(3):389–401
14. Scrucca L, Santucci A, Aversa F (2007) Competing risk analysis using R: an easy guide for clinicians. Bone Marrow Transplant 40(4):381–387
15. Scrucca L, Santucci A, Aversa F (2010) Regression modeling of competing risk using R: an in depth guide for clinicians. Bone Marrow Transplant 45(9):1388–1395
16. Arcenas R, Widen RH (2002) Epstein-Barr virus reactivation after superinfection of the BJAB-B1 and P3HR-1 cell lines with cytomegalovirus. BMC Microbiol 2:20
17. Zallio F, Primon V, Tamiazzo S, Pini M, Baraldi A, Corsetti MT, Gotta F, Bertassello C, Salvi F, Rocchetti A, Levis A (2013) Epstein-Barr virus reactivation in allogeneic stem cell transplantation is highly related to cytomegalovirus reactivation. Clin Transpl 27(4):E491–E497
18. Papadopoulou A, Gerdemann U, Katari UL, Tzannou I, Liu H, Martinez C, Leung K, Carrum G, Gee AP, Vera JF, Krance RA, Brenner MK, Rooney CM, Heslop HE, Leen AM (2014) Activity of broad-spectrum T cells as treatment for AdV, EBV, CMV, BKV, and HHV6 infections after HSCT. Sci Transl Med 6(242):242ra83
19. Janeczko M, Mielcarek M, Rybka B, Ryczan-Krawczyk R, Noworolska-Sauren D, Kałwak K (2016) Immune recovery and the risk of CMV/ EBV reactivation in children post allogeneic haematopoietic stem cell transplantation. Cent Eur J Immunol 41(3):287–296
20. Klyuchnikov E, Asenova S, Kern W, Kilinc G, Ayuk F, Wiedemann B, Lioznov M, Freiberger P, Zalyalov Y, Zander AR, Kröger N, Bacher U (2010) Post-transplant immune reconstitution after unrelated allogeneic stem cell transplant in patients with acute myeloid leukemia. Leuk Lymphoma 51(8):1450–1463
21. Styczynski J, Einsele H, Gil L, Ljungman P (2009) Outcome of treatment of Epstein-Barr viruserelated post-transplant lymphoproliferative disorder in hematopoietic stem cell recipients: a comprehensive review of reported cases. Transpl Infect Dis 11(5):383–392
22. Styczynski J, van der Velden W, Fox CP, Engelhard D, de la Camara R, Cordonnier C, Ljungman P; Sixth European Conference on Infections in Leukemia, a joint venture of the Infectious Diseases Working Party of the European Society of Blood and Marrow Transplantation (EBMT-IDWP), the Infectious Diseases Group of the European Organization for Research and Treatment of Cancer (EORTC-IDG), the International Immunocompromised Host Society (ICHS) and the European Leukemia Net (ELN) (2016) Management of Epstein-Barr Virus infections and post-transplant lymphoproliferative disorders in patients after allogeneic hematopoietic stem cell transplantation: Sixth European Conference on Infections in Leukemia (ECIL-6) guidelines. Haematologica 101(7):803–811
23. Xu LP, Zhang CL, Mo XD, Zhang XH, Chen H, Han W, Chen YH, Wang Y, Yan CH, Wang JZ, Wang FR, Zhao T, Liu YR, Liu KY, Huang XJ (2015) Epstein-Barr virus-related post-transplantation lymphoproliferative disorder after unmanipulated human leukocyte antigen haploidentical hematopoietic stem cell transplantation: incidence, risk factors, treatment, and clinical outcomes. Biol Blood Marrow Transplant 21(12):2185–2191
24. Abruzzo LV, Rosales CM, Medeiros LJ, Vega F, Luthra R, Manning JT, Keating MJ, Jones D (2002) Epstein-Barr virus-positive B-cell lymphoproliferative disorders arising in immunodeficient patients previously treated with fludarabine for low-grade B-cell neoplasms. Am J Surg Pathol 26(5):630–636
25. Lange A, Klimczak A, Dlubek D, Dybko J (2003) B-cell lymphoproliferative syndrome and peripheral blood CD20+ cells expansion after hematopoietic stem cell transplantation: association with fludarabine and anti-thymocyte globulin containing conditioning regimen. Transplant Proc 35(8):3093–3095
26. Dharnidharka VR, Tejani AH, Ho PL, Harmon WE (2002) Post-transplant lymphoproliferative disorder in the United States: young Caucasian males are at highest risk. Am J Transplant 2(10):993–998