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Prognostic impact of Epstein-Barr virus serostatus in patients with nonmalignant hematological disorders undergoing allogeneic hematopoietic cell transplantation: the study of Infectious Diseases Working Party of the European Society for Blood and Marrow Transplantation

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Jan Styczynski[1*], Gloria Tridello[2], Lidia Gil[3], Per Ljungman[4], Malgorzata Mikulska[5], Steffie van der Werf[6], Nina Simone Knelange[6], Diana Averbuch[7], Gerard Socié[8], Hendrik Veelken[9], Jean-Hugues Dalle[10], Mahmoud Aljurf[11], Alphan Kupesiz[12], Yves Bertrand[13], Abdelghani Tbakhi[14], Boris Afanasyev[15], Bruno Lioure[16], Hélène Labussière-Wallet[17], Xavier Poiré[18], Johan Maertens[19], Eefke Petersen[20], Patrice Chevallier[21], Noel Milpied[22], John A. Snowden[23], Ibrahim Yakoub-Agha[24], Jan Cornelissen[25], Nicolaas Schaap[26], Carlo Dufour[27], Regis Peffault de Latour[8], Arjan Lankester[9], Simone Cesaro[2] on behalf of the Infectious Diseases Working Party EBMT

[1] *Department of Pediatric Hematology and Oncology, Jurasz University Hospital, Collegium Medicum UMK Toruń, Bydgoszcz, Poland*
[2] *Pediatric Hematology Oncology, Ospedale Donna Bambino, Azienda Ospedaliera Universitaria Integrata, Verona, Italy*
[3] *Department of Hematology, Medical University, Poznań, Poland*
[4] *Department of Cellular Therapy and Allogeneic Stem Cell Transplantation, Karolinska University Hospital, and Division of Hematology, Department of Medicine Huddinge, Karolinska Institutet, Stockholm, Sweden*
[5] *Division of Infectious Diseases, Department of Health Sciences, University of Genova, Ospedale Policlinco San Martino, Genova, Italy*
[6] *EBMT Data Office, Leiden, Netherlands*
[7] *Hadassah University Hospital, Jerusalem, Israel*
[8] *Hopital St. Louis, Paris, France*
[9] *Leiden University Hospital, Leiden, Netherlands*
[10] *Hôpital Robert Debre, Paris, France*
[11] *King Faisal Specialist Hospital & Research Centre, Riyadh Saudi Arabia*
[12] *Akdeniz University Medical School, Antalya, Turkey*
[13] *Institut d`Hematologie et d`Oncologie Pediatrique, Lyon, France*
[14] *King Hussein Cancer Centre, Amman, Jordan*
[15] *First State Pavlov Medical University of St. Petersburg, St. Petersburg, Russia*
[16] *Techniciens d`Etude Clinique suivi de patients greffes, Strasbourg, France*
[17] *Centre Hospitalier Lyon Sud, Lyon, France*
[18] *Cliniques Universitaires St. Luc, Brussels, Belgium*
[19] *University Hospital Gasthuisberg, Leuven, Belgium*
[20] *University Medical Centre, Utrecht, Netherlands*
[21] *CHU Nantes, Nantes, France*
[22] *CHU Bordeaux, Pessac, France*
[23] *Sheffield Teaching Hospitals NHS Trust, Sheffield, UK*
[24] *CHU de Lille, LIRIC, INSERM U995, Université de Lille, 59000 Lille, France*
[25] *Erasmus MC Cancer Institute, Rotterdan, Netherlands*
[26] *Radboud University Medical Centre, Nijmegen, Netherlands*
[27] *Instituto Giannina Gaslini, Genova, Italy*

Abstract

Background: In patients with acute leukemia, lymphoma and chronic malignancies, donor and/or recipient Epstein-Barr virus (EBV) seropositive status increases the risk of development of chronic graft-versus-host disease (cGVHD) after allo-hematopoietic cell transplantation (allo-HCT), while it has no influence on other transplant outcomes. No data are available on the impact of EBV serostatus on transplant outcomes in patients with nonmalignant hematological disorders. Objective: We analyzed the influence of the recipient's (R) and donor's (D) EBV serostatus on transplant outcomes (overall survival (OS); relapse-free survival (RFS); relapse incidence (RI); nonrelapse mortality (NRM); acute graft-versus-host disease (aGVHD); cGVHD) in patients with nonmalignant hematological disorders undergoing allo-HCT. Patients and Methods: A total of 2,355 allo-HCTs performed between 1997 and 2016 for acquired bone marrow failure or hemoglobinopathies were included in this retrospective Registry megafile Infectious Diseases Working Party of the European Society of Blood and Marrow Transplantation (IDWP-EBMT) study. Results: *Demographics*: The median age of recipient was 17.7 years (range: 0–77), and 50.8% were children. 79.0% of recipients and 75.4% of donors were EBV-seropositive. 67.8% had HCT from a matched family donor, 4.6% from a mismatched family donor, and 27.6% from an unrelated donor (UD). T-cell depletion was performed *in vivo* and *ex vivo* in 82.2% and 6.6% of patients, respectively. Conditioning regimen was myeloablative in 63.7% and reduced intensity conditioning (RIC) in 36.3% of patients. The median follow-up was 4.7 years. *Transplant outcomes*: EBV-seropositive recipients in comparison with EBV-seronegative recipients had lower OS (85.4% vs. 88.4%, $p = 0.035$) and higher NRM (10.0% vs. 6.4%, $p = 0.018$). No other significant differences were found for: RI, RFS, and aGVHD or cGVHD with respect to EBV pretransplant serostatus donor and/or recipient. *Multivariate analysis*: A trend toward higher risk of development of cGVHD (HR = 1.31; $p = 0.081$) and better survival (HR = 0.78; $p = 0.087$) in allo-HCT from EBV-seropositive donors was found. Allo-HCT in EBV-seropositive recipients had a trend toward lower risk of development of cGVHD (HR = 0.75; $p = 0.065$). When four subgroups (R–/D–, R–/D+, R+/D–, R+/D+ EBV serology) were analyzed, the EBV serostatus had no significant impact on OS, RFS, RI, NRM and development of aGVHD or cGVHD. Conclusions: Allo-HCT from EBV-seropositive versus EBV-seronegative donors are at 31% higher risk of cGVHD in patients with nonmalignant hematological disorders undergoing allo-HCT; however this difference is nonsignificant in multivariate analysis.

Keywords:
hematopoietic cell transplant, graft-versus-host disease, Epstein-Barr virus, overall survival

Introduction

Epstein-Barr virus (EBV) is one of the most common human viruses, and the prevalence in adults is about 84% [1, 2]. In general, the incidence of EBV positivity in population is increasing with age and decreasing by calendar year. EBV is responsible for the development of a number of various diseases, such as post-transplant lymphoproliferative disorder (PTLD) and several other end-organ diseases, both after hematopoietic cell transplantation (HCT) and solid organ transplants (SOT) [3]. EBV serostatus of donor (D) and recipient (R) is a strong risk factor for the development of PTLD [3, 4]. Continuous progress in diagnosis and therapy resulted in an increase in the survival rate after PTLD from 16% before the year 2000 up to 70% in 2013 [5, 6].

* Corresponding author: Jan Styczyński, Department of Pediatric Hematology and Oncology; Collegium Medicum, Nicolaus Copernicus University in Torun, Skłodowskiej-Curie 9; 85-094 Bydgoszcz, Poland, phone: +48 52 5854860, fax: +48 52 5854087, e-mail: jstyczynski@cm.umk.pl

Recently, it was shown in two studies that EBV is associated with the increased risk of chronic graft-versus-host disease (cGVHD). In patients with acute leukemias, donor EBV-seropositivity significantly increases the risk of chronic, and to lesser extent acute graft-versus--host disease (aGVHD) after allo-HCT, with no significant association with other transplant outcomes [1]. In patients with lymphomas or chronic malignancies undergoing allo-HCT, it has been shown that the risk of development of cGVHD was increased for EBV R+/D+, R+/D−, and R−/D+ in comparison with R−/D− transplants, thus an EBV-negative recipient with lymphoma or chronic malignancy can benefit from the selection of an EBV-negative donor in the context of cGVHD, while there are no preferences in donor EBV serostatus for EBV-seropositive recipient [7].

Since so far no data were available on the role of serostatus of EBV on outcome of HCT in patients with nonmalignant hematological diseases, the objective of the study was to analyze the impact of EBV pre-transplant recipient and/or donor serostatus on transplant outcomes.

Patients and methods

Study design

A total number of 2,355 patients with nonmalignant hematological diseases who underwent allo-HCT between 1997 and 2016 and reported to the EBMT Registry were included in this retrospective study performed by Infectious Diseases Working Party of the European Society of Blood and Marrow Transplantation (IDWP-EBMT). Diagnosis included acquired bone marrow failure (70.1%) or hemoglobinopathies (29.9%). The following criteria of inclusion for the study were used based on full data availability on first transplant, type of conditioning, source of stem cells (including cord blood), and recipient and donor EBV serostatus. The study was performed within EBMT according to the Declaration of Helsinki.

Definitions

Overall survival (OS) was calculated as time from allo-HCT to death. Death from any cause was regarded as event for OS. Relapse or death from any cause were analyzed as an event for relapse-free survival (RFS), hereby defined as survival with no evidence of relapse or progression. Relapse was considered as the reappearance of the primary disease. Relapse incidence (RI) was calculated with relapse of the primary disease (in this setting: graft rejection) as event and death without relapse as a competing event. Nonrelapse mortality (NRM) was defined as death without evidence of relapse or progression, with relapse being a competing event. aGVHD was graded according to classical criteria by Glucksberg et al. [8], and cGVHD was classified as extensive or limited. *De novo* cGVHD was defined for cGVHD occurring without previous aGVHD.

Statistical analyses

The primary endpoint of the study was the probability of OS, whereas RI, RFS, NRM, aGVHD, cGVHD, and *de novo* cGVHD were regarded to be the secondary endpoints. The cumulative incidences of aGVHD, cGVHD, and *de novo* cGVHD were estimated for the respective type of GVHD, considering the event of interest and death without GVHD being the competing event. The cumulative incidences of RI, NRM, and GVHD were computed in a competing risks analysis, and the Gray test was being used to compare the groups with the delta method for confidence intervals. OS and RFS were analyzed with the Kaplan-Meier method; the log-rank test was used for univariate comparisons, while the Greenwood formula for computing confidence intervals.

Categorical variables were compared with the use of Chi-squared test or Fisher's exact test. The proportional hazard assumption was verified using graphical methods [9, 10]. The uni- and multivariate analyses of prognostic factors were performed with the use of a Cox proportional hazards model to estimate hazard risk (HR) effect of variables on OS and RFS. Factors analyzed are listed in table I. Cause-specific HRs were investigated for RI, NRM, aGVHD, cGVHD, and *de novo* cGVHD [11]. Variables with p-value < 0.15 in univariate analysis were included in multivariate model. All tests were two-sided. For multiple subgroup comparisons, the Bonferroni correction was applied. The inverted Kaplan-Meier method was used to calculate the median follow-up [12]. Analyses were performed by using the statistical package SAS (SAS Institute Inc., Cary, NC, USA) version 9.4.

Results

Demographics

Patient characteristics are presented in table I. The majority of donors (74.4%) and recipients (79.2%) were EBV-seropositive. Both donor and recipient were seropositive in 1,541 cases (65.4%), while conversely both donor and recipient were seronegative in 276 cases (11.7%). With respect to the type of transplant, 66.7% had HCT from a matched family donor (MFD), 4.4% patients from a mismatched family donor (MMFD), and 28.9% HCT from an UD. The stem cell source included bone marrow ± cord blood (BM ± CB, 75.8%) or peripheral blood ± cord blood ± bone marrow (PB ± CB, 24.2%). T-cell depletion was used *in vivo* in 82.2%, while *ex vivo* in 6.4% of patients. Myeloablative conditioning (MAC) was used in 63.2%, and reduced-intensity regimen (RIC) in 36.8%. The median follow-up was 4.6 (95%CI, 4.4–4.8) years.

Univariate analysis: EBV serostatus and transplant outcomes

Transplants of EBV-seronegative recipients of grafts from EBV--seronegative donors (EBV R−/D−) were of nonsignificant benefit when compared with EBV-seropositive recipients of grafts from any donors (EBV R+/D±) or EBV-seronegative recipients of grafts from EBV-seropositive donors for OS, RFS, NRM, and aGVHD (Tab. II, Fig. 1).

EBV-seropositive recipients of grafts from EBV-seropositive donors (EBV R+/D+) had nonsignificant inferior outcomes when compared with EBV-seronegative recipients of grafts from EBV-seronegative donors (EBV R−/D−): inferior OS (85.4% vs. 89.3%), inferior RFS (86.6% vs. 88.9%), increased NRM (10.2% vs. 5.8%), increased

Table I. Characteristics of patients and transplant

	EBV in patient and donor				Total (N = 2,355)
	–/– (N = 276)	–/+ (N = 213)	+/– (N = 325)	+/+ (N = 1,541)	
	N (%)	N (%)	N (%)	N (%)	N (%)
Sex					
Male	148 (53.6)	103 (48.4)	162 (49.8)	831 (53.9)	1,244 (52.8)
Female	128 (46.4)	110 (51.6)	163 (50.2)	710 (46.1)	1,111 (47.2)
Age at this treatment (years)					
Median	14.0	8.6	17.2	19.3	17.3
Range	0.5–69.8	0.5–62.8	1.0–67.8	0.9–77.7	0.5–77.7
N obs	276	213	325	1,541	2,355
Age classes					
<18 years	171 (62.0)	161 (75.6)	171 (52.6)	714 (46.3)	1,217 (51.7)
≥18 years	105 (38.0)	52 (24.4)	154 (47.4)	827 (53.7)	1,138 (48.3)
Diagnosis					
Acquired bone marrow failure	184 (66.7)	104 (48.8)	237 (72.9)	1,127 (73.1)	1,652 (70.1)
Hemoglobinopathies	92 (33.3)	109 (51.2)	88 (27.1)	414 (26.9)	703 (29.9)
Interval between diagnosis and HCT					
Median	8.2	17.0	11.2	11.4	11.3
Range	0.3–293.9	0.4–409.3	0.4–348.6	0.1–540.7	0.1–540.7
N obs	276	213	325	1,541	2,355
Donor sex (N = 2,335/2,355)					
Male	130 (47.1)	128 (60.7)	188 (58.8)	867 (56.7)	1,313 (56.2)
Female	146 (52.9)	83 (39.3)	132 (41.3)	661 (43.3)	1,022 (43.8)
Age of the donor (N = 2,255/2,355)					
Median	17.0	20.6	16.1	24.9	23.2
Range	0.0–58.5	0.0–65.9	0.0–66.8	0.0–76.2	0.0–76.2
N obs	261	207	309	1,478	2,255
Recipient male – donor female match (2,335/2,355)					
Other combinations	196 (71.0)	175 (82.9)	250 (78.1)	1,189 (77.8)	1,810 (77.5)
Recipient male – donor female	80 (29.0)	36 (17.1)	70 (21.9)	339 (22.2)	525 (22.5)
Recipient–donor match					
Recipient male – donor male	68 (24.6)	66 (31.3)	92 (28.8)	483 (31.6)	709 (30.4)
Recipient male – donor female	80 (29.0)	36 (17.1)	70 (21.9)	339 (22.2)	525 (22.5)
Recipient female – donor male	62 (22.5)	62 (29.4)	96 (30.0)	384 (25.1)	604 (25.9)
Recipient female – donor female	66 (23.9)	47 (22.3)	62 (19.4)	322 (21.1)	497 (21.3)
CMV in patient and donor					
-/-	117 (42.4)	53 (24.9)	90 (27.7)	333 (21.6)	593 (25.2)
-/+	29 (10.5)	46 (21.6)	15 (4.6)	148 (9.6)	238 (10.1)
±	38 (13.8)	30 (14.1)	101 (31.1)	308 (20.0)	477 (20.3)
+/+	92 (33.3)	84 (39.4)	119 (36.6)	752 (48.8)	1,047 (44.5)
Stem cell source					
BM	175 (63.4)	159 (74.6)	218 (67.1)	1,122 (72.8)	1,674 (71.1)
PB	47 (17.0)	40 (18.8)	52 (16.0)	357 (23.2)	496 (21.1)
BM + PB	22 (8.0)	3 (1.4)	0 (0.0)	6 (0.4)	31 (1.3)
CB	9 (3.3)	11 (5.2)	39 (12.0)	47 (3.0)	106 (4.5)
BM + CB	8 (2.9)	0 (0.0)	16 (4.9)	8 (0.5)	32 (1.4)
PB + CB	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.1)	1 (0.0)
BM + PB + CB	15 (5.4)	0 (0.0)	0 (0.0)	0 (0.0)	15 (0.6)

continued Table I.

	EBV in patient and donor				Total (N = 2,355)
	–/– (N = 276)	–/+ (N = 213)	+/– (N = 325)	+/+ (N = 1,541)	
	N (%)	N (%)	N (%)	N (%)	N (%)
Donor type					
Sibling	215 (77.9)	139 (65.3)	219 (67.4)	997 (64.7)	1,570 (66.7)
Mismatched relative	24 (8.7)	8 (3.8)	8 (2.5)	64 (4.2)	104 (4.4)
Unrelated	37 (13.4)	66 (31.0)	98 (30.2)	480 (31.1)	681 (28.9)
HLA match					
Identical sibling	210 (76.1)	128 (60.1)	211 (64.9)	948 (61.5)	1,497 (63.6)
Syngeneic	2 (0.7)	0 (0.0)	2 (0.6)	16 (1.0)	20 (0.8)
Matched other relative	3 (1.1)	11 (5.2)	6 (1.8)	33 (2.1)	53 (2.3)
Matched unrelated	11 (4.0)	13 (6.1)	21 (6.5)	102 (6.6)	147 (6.2)
Mismatched relative	24 (8.7)	8 (3.8)	8 (2.5)	64 (4.2)	104 (4.4)
Mismatched unrelated	3 (1.1)	7 (3.3)	21 (6.5)	51 (3.3)	82 (3.5)
Unrelated	23 (8.3)	46 (21.6)	56 (17.2)	327 (21.2)	452 (19.2)
Ex-vivo T-cell depletion for HCT					
No	263 (95.3)	197 (92.5)	315 (96.9)	1,430 (92.8)	2,205 (93.6)
Yes	13 (4.7)	16 (7.5)	10 (3.1)	111 (7.2)	150 (6.4)
In-vivo T-cell depletion for HCT (N = 2,354/2,355)					
No	95 (34.4)	47 (22.1)	70 (21.5)	206 (13.4)	418 (17.8)
Yes	181 (65.6)	166 (77.9)	255 (78.5)	1,334 (86.6)	1,936 (82.2)
Intensity of conditioning					
Standard	230 (83.3)	152 (71.4)	200 (61.5)	907 (58.9)	1,489 (63.2)
Reduced	46 (16.7)	61 (28.6)	125 (38.5)	634 (41.1)	866 (36.8)
Year of this treatment					
Median	2009	2010	2010	2010	2010
Range	1997–2016	1997–2016	1997–2016	1997–2016	1997–2016
N obs	276	213	325	1,541	2,355

HCT – hematopoietic cell transplantation; PB – peripheral blood; BM – bone marrow; CB – cord blood; N – number of patients

aGVHD incidence (14.3% vs. 13.1%), and increased cGVHD incidence (17.3% vs. 16.6%). EBV-seropositive versus seronegative donor (D+ vs. D−) transplants had comparable outcomes; however, there was a trend toward higher cGVHD in EBV-seropositive donor transplants (Tab. II).

Pre-transplant recipient EBV-seronegativity had favorable significant influence on higher OS (p = 0.035) and lower incidence of NMR (p = 0.017), however a trend was observed toward higher RI (p = 0.078) in comparison to EBV-seropositive status (Tab. II).

aGVHD grade II–IV was diagnosed in 320 (14.3%) patients. Cumulative incidence of aGVHD was 13.1% in EBV R−/D− and 14.3% in R+/D+ cases (p = ns). The highest aGVHD was observed after R−/D+ transplants (16.0%). The 100-day cumulative incidence of grade II-IV aGVHD was comparable for EBV-seropositive versus seronegative donors (14.5% vs. 13.7%, p = ns) (Tab. II). Also, no impact of recipient EBV serostatus was observed.

cGVHD was diagnosed in 331 (16.9%) of the 1,958 evaluable patients. The number of cGVHD cases following aGVHD was 95 (4.9%) and *de novo* cGVHD events was 236 (12.0%), as computed on 1,958 patients with available data for a/cGVHD. The highest cGVHD and *de novo* cGVHD was observed after R−D+ transplants (18.9% and 13.7%, respectively). There was a trend toward higher incidence of cGVHD in EBV D+ transplants in comparison to EBV D− transplants (p = 0.106); the impact of donor EBV serostatus was not significant for *de novo* cGVHD (Tab. II). No impact of recipient EBV serostatus was observed on the development of aGVHD or cGVHD. The cumulative incidences of cGVHD did not differ taking into account primary disease and EBV serostatus (data not shown).

Multivariate analysis

No impact of donor/recipient EBV serostatus on OS, RI, NRM, RFS, and aGVHD was shown (Tab. III). A trend was observed toward higher risk of cGVHD in HCT from EBV-seropositive donors (HR = 1.31; 95% CI = 0.97–1.78; p = 0.0810), and lower risk of cGVHD (HR = 0.75; 95% CI = 0.56–1.02; p = 0.0657) and *de novo* cGVHD (HR = 0.72; 95% CI = 0.50–1.02; p = 0.0633) in case of EBV-seropositive recipients (Tab. III).

Factors significantly contributing to an increased risk of aGVHD were: increasing age of donor and recipient (continuous variable), female

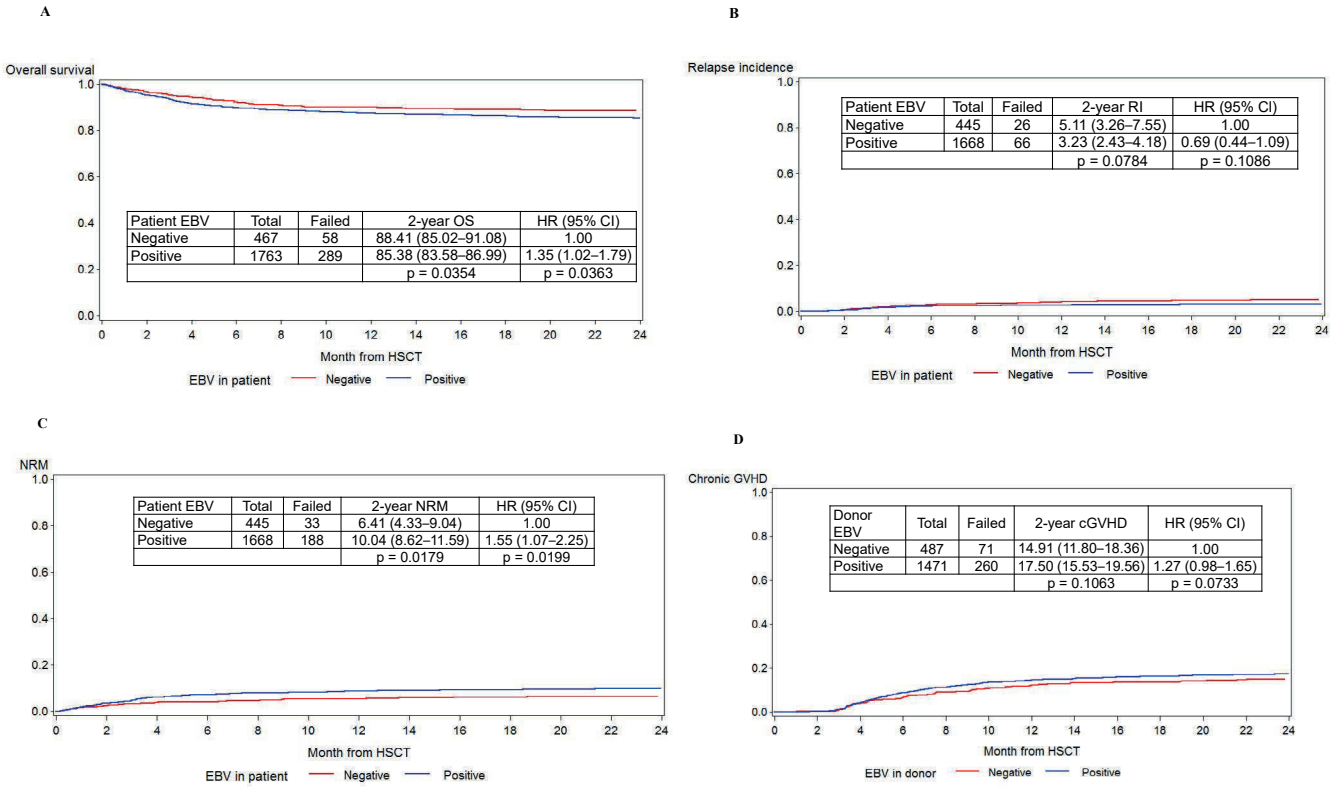

Fig. 1. Impact of recipient (R)/donor (D) EBV serostatus on the 2-year transplant outcomes: (A) overall survival (OS); (B) relapse incidence (RI); (C) nonrelapse mortality (NRM); and (D) cumulative incidence of cGVHD

Table II. Univariate analysis of an impact of EBV serostatus on overall survival (OS), relapse-free survival (RFS), relapse incidence (RI), nonrelapse mortality (NRM), acute and chronic GVHD (in% and 95%CI)

EBV Serostatus (N = 2,355)	OS	RFS	RI†	NRM†	aGVHD	cGVHD†	De Novo cGVHD†
Recipient/Donor							
Negative/Negative	89.30 (84.76–92.55)	88.89 (84.44–92.54)	5.29 (2.87–8.78)	5.82 (3.32–9.29)	13.13 (9.40–17.49)	16.60 (11.94–21.93)	13.10 (8.94–18.07)
Negative/Positive	87.17 (81.44–91.22)	87.85 (82.59–92.13)	4.92 (2.41–8.75)	7.23 (4.03–11.66)	16.04 (11.33–21.49)	18.85 (13.34–25.11)	13.73 (9.01–19.44)
Positive/Negative	85.45 (80.60–89.16)	87.28 (82.88–91.00)	3.55 (1.75–6.38)	9.17 (6.06–13.07)	14.23 (10.44–18.59)	13.51 (9.51–18.21)	9.45 (6.11–13.64)
Positive/Positive	85.37 (83.39–87.12)	86.62 (84.72–88.39)	3.16 (2.32–4.21)	10.22 (8.66–11.92)	14.28 (12.55–16.12)	17.31 (15.22–19.51)	11.90 (10.14–13.82)
p-value	0.1953	0.7412	0.2702	0.1217	0.8585	0.2577	0.3285
Donor							
Negative	87.30 (84.10–89.89)	88.08 (85.03–90.76)	4.36 (2.78–6.46)	7.56 (5.46–10.10)	13.69 (10.97–16.72)	14.91 (11.80–18.36)	11.12 (8.41–14.25)
Positive	85.58 (83.75–87.23)	86.78 (85.01–88.44)	3.37 (2.54–4.37)	9.85 (8.41–11.42)	14.49 (12.85–16.22)	17.50 (15.53–19.56)	12.13 (10.46–13.93)
p-value	0.4031	0.8833	0.1356	0.2625	0.6081	0.1063	0.2749
Recipient							
Negative	88.41 (85.02–91.08)	88.48 (85.18–91.34)	5.11 (3.26–7.55)	6.41 (4.33–9.04)	14.38 (11.37–17.73)	17.53 (13.90–21.51)	13.33 (10.12–16.97)
Positive	85.38 (83.58–86.99)	86.73 (85.00–88.35)	3.23 (2.43–4.18)	10.04 (8.62–11.59)	14.27 (12.68–15.95)	16.68 (14.80–18.66)	11.50 (9.91–13.22)
p-value	0.0354	0.2892	0.0784	0.0179	0.9813	0.7059	0.4058

*Two-year probabilities (%, 95% CI) are shown for OS, RFS, RI and NRM, cGVHD, de novo GVHD; 100-day probabilities are shown for aGVHD (grade II-IV), * probabilities were obtained using the Kaplan-Meier method, log-rank test was used to compare groups, † probabilities were obtained using the Cumulative Incidence method, Gray test was used to compare groups; OS – overall survival; RFS – relapse-free survival; RI – relapse incidence; NRM – nonrelapse mortality; aGVHD – acute graft-versus-host disease; cGVHD – chronic GVHD*

Table III. Multivariate analysis of risk factors

	OS *p*-value	RI *p*-value	NRM§ *p*-value	RFS§ *p*-value	aGVHD§ *p*-value	cGVHD§ *p*-value	de novo cGVHD§ *p*-value
Variables	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Donor EBV (Positive vs. Negative)#	0.78 (0.59–1.04) **0.0870**	0.76 (0.46–1.23) 0.2608	0.86 (0.60–1.24) 0.4239	0.80 (0.60–1.08) 0.1420	1.12 (0.83–1.51) 0.4771	1.31 (0.97–1.78) **0.0810**	1.26 (0.89–1.80) 0.1991
Recipient EBV (Positive vs. Negative)#	1.08 (0.78–1.48) 0.6501	0.74 (0.45–1.23) 0.2423	1.17 (0.77–1.79) 0.4692	0.94 (0.68–1.29) 0.6881	0.97 (0.72–1.31) 0.8453	0.75 (0.56–1.02) **0.0657**	0.72 (0.50–1.02) **0.0633**
Age at HCT (10 years effect)	1.29 (1.20–1.38) <0.0001	ns	1.29 (1.18–1.42) <0.0001	1.22 (1.12–1.32) <0.0001	ns	1.11 (1.02–1.21) 0.0172	1.16 (1.05–1.28) 0.0051
Donor sex (Female vs. Male)	ns	ns	ns	ns	ns	1.50 (1.20–1.88) 0.0004	1.37 (1.05–1.77) 0.0190
Donor age (10 years effect)	1.22 (1.12–1.33) <0.0001	ns	1.27 (1.14–1.42) <0.0001	1.18 (1.07–1.30) 0.0005	1.09 (1.00–1.19) 0.0396	1.16 (1.06–1.28) 0.0018	1.13 (1.01–1.26) 0.0371
Stem cell source (BM vs. PB)	ns	ns	0.71 (0.53–0.96) 0.0238	0.75 (0.59–0.97) 0.0285	ns	0.68 (0.53–0.89) 0.0041	ns
Donor (Mismatched relative vs. Sibling)	2.76 (1.84–4.12) <0.0001	ns	2.77 (1.62–4.72) 0.0002	2.18 (1.37–3.47) 0.0010	2.16 (1.33–3.50) 0.0018	1.90 (1.11–3.27) 0.0201	ns
Donor (Unrelated vs. Sibling)	1.87 (1.49–2.35) <0.0001	ns	2.14 (1.60–2.86) <0.0001	1.50 (1.17–1.91) 0.0012	2.44 (1.87–3.18) <0.0001	1.79 (1.38–2.33) <0.0001	ns
Ex-vivo T-cell depletion (yes vs. no)	1.60 (1.14–2.23) 0.0059	ns	ns	ns		0.45 (0.26–0.79) 0.0053	ns
In vivo T-cell depletion (yes vs. no)	ns	ns	ns	ns	0.69 (0.51–0.94) 0.0167	0.64 (0.48-0.85) 0.0018	0.67 (0.49–0.92)
Conditioning regimen (RIC vs. Standard)	ns	ns	0.69 (0.51–0.93) 0.0161	ns	ns	ns	ns
Year of HCT	ns	0.95 (0.91–0.99) 0.0176	ns	ns	0.96 (0.94–0.99) 0.0011	0.96 (0.94–0.98) 0.0006	ns

Shown are hazard ratios with 95% confidence interval (n = 2,230); p-value for the overall comparison; ns – not significant; Hazard ratio obtain from the Cox model were reported; § Cause-specific hazards were reported; # Bonferroni adjusted p-values are considered for Recipient (R)/donor(D) EBV serostatus, recipient (R)/donor(D) CMV serostatus, and donor type, OS – overall survival; RFS – relapse-free survival; RI – relapse incidence; NRM – nonrelapse mortality; aGVHD – acute graft-versus-host disease; cGVHD – chronic GVHD

donor for male recipient, stem cell source from PB, mismatched donor, UD, no *in vivo* T-cell depletion, and earlier calendar year of transplant. Factors increasing risk of cGVHD included: increasing age of donor and recipient (continuous variable), female donor for male recipient, stem cell source from PB, no T-cell depletion (both *ex vivo* and *in vivo*), MMFD, UD, and earlier calendar year of transplant (Tab. III).

Discussion

This study was aimed to show the influence of donor/recipient EBV serostatus on transplant outcomes of patients with nonmalignant hematological disorders. We analyzed 2,55 patients with acquired bone marrow failure or hemoglobinopathies. Overall, no significant impact of donor and/or recipient EBV-seropositivity was shown on any transplant outcome. Only a trend toward increased risk of cGVHD was observed in the case of donor EBV-seropositivity (HR = 1.31, $p = 0.08$).

This is the third part of the study focused on the impact of EBV donor and recipient serostatus on transplant outcomes. In the first study of 11,364 patients with acute leukemia after allo-HCT, we have proven that donor EBV-seropositivity increases the risk of aGVHD and cGVHD [1]. A 1.4-fold higher risk of cGVHD was observed in patients who received grafts from EBV-seropositive versus EBV-seronegative donors. The evidence that donor EBV-seropositivity increases the risk of cGVHD, and to a lesser extent also aGVHD, was a new and striking finding for patients with acute leukemias. No impact of EBV serostatus was found for other transplant outcomes.

In the second study of 12,931 patients with lymphomas or chronic malignancies undergoing allo-HCT, we have shown the impact of donor EBV-seropositivity on the development of cGVHD also in patients with lymphomas or chronic hematological malignant diseases [7]. However, in these group of patients, the role of recipient EBV-seropositivity was also shown, and in comparison with D−/R− EBV serostatus, all other combinations (D−/R+, D+/R−, D+/R+) were associated with 1.21–1.26-fold increased risk of cGVHD. Nevertheless, the role of recipient EBV in pathogenesis of lymphomas should be considered. Again, no impact of EBV serostatus donor and/or recipient for other transplant outcomes was shown in multivariate analysis. Thus, the EBV-seronegative recipient with chronic malignancy or lymphoma undergoing allo-HCT can possibly benefit from selection of an EBV-seronegative donor in the context of cGVHD, while there is no preference in donor EBV serostatus for EBV-positive recipient.

In the present study of patients with nonmalignant hematological diseases, we observed the comparable increased risk of cGVHD in case of donor EBV-seropositivity, as it was found in previous two studies. In this study, however, only a trend was observed, but not statistical significance. On the other hand, the present study included

2,355 patients, relatively smaller number than in previous reports. One can speculate that if the number of patients was higher as in the two other studies, we could have reached statistical significance. This limitation can be probably overcome in the future when more patients will be included in such an analysis.

Summarizing the results of 26,650 patients with various hematological diseases undergoing allo-HCT, reported in these three studies, we have found that the donor EBV seropositivity is associated with the development of cGVHD. This rises the potential possibility to prevent or treat cGVHD by controlling EBV infection.

The herpesvirus EBV is associated in the HCT setting with the development of PTLD which is almost exclusively of donor origin [13, 14]. Finding a role of donor EBV serostatus on the development of both cGHVD and PTLD rises the possibility of potential benefit of the selection of an EBV-seronegative donor, what might potentially reduce the risk of these two severe post-transplant complications. However, given the high prevalence of EBV, finding the EBV-seronegative adult donor would be highly challenging, so such a recommendation is rather more academic than practical [7]. The management recommended against EBV-PTLD in allo-HCT recipients is screening and monitoring for EBV by PCR, and pre-emptive use of rituximab [15].

We have shown previously, that despite the impact of donor EBV-seropositivity on GVHD no increased GVHD-related deaths rate, and in consequence no effect on OS, RI, RFS, and NRM was observed in acute leukemia patients [1]. In another study, we have found the negative influence of EBV serology on transplant outcomes in lymphomas and chronic hematological malignancies in univariate analysis [7]. OS and RFS were decreased in EBV-seropositive recipients, while donor EBV serology had no impact on OS and RFS. This adverse effect of EBV cannot be fully overcome by allo-HCT, as both OS and RFS did not differ between transplants from EBV-seropositive versus EBV-seronegative donors. Since children were more likely to be the R−/D− patients than R+/D+ combination (Tab. I), this could explain the impact in univariate analysis on OS/RFS while the cGVHD effect was not dependent on age. The role of EBV in pathogenesis of lymphomas possibly did not influence transplant outcomes negatively, since in multivariate analysis myeloid but not lymphoid malignancies had negative impact on OS, RI, RFS, and NRM. In both studies, neither donor nor recipient EBV serology had influence on NRM. This underlines that variety of pre- and post-transplant factors contributing to final transplant outcomes.

The role of B-cell proliferation in pathogenesis of cGVHD is currently well known [16]. On the other hand, the efficacy of anti-B-cell approach with rituximab in therapy of steroid-refractory cGVHD was shown in meta-analysis [17]. This efficacy was also confirmed in Phase II study in first line therapy for cGVHD [18]. The effect of EBV was more obvious in the development of cGVHD rather than aGVHD, as it is related to B-cell recovery occurring usually after day +100.

Apart from the relatively low number of patients, our study has some other limitations. No data on pretransplant use of rituximab was documented. Prolonged immunosuppressive prophylaxis in patients with bone marrow failure theoretically could influence the development of GVHD. However, we did not find differences between patients with acquired bone marrow failure and hemoglobinopathies with respect to a/cGVHD. Also, the selection of patients was based mainly on the availability of data on recipient and donor EBV serostatus, so any specific transplant strategies could not be analyzed.

In conclusion, we were unable to show the impact of donor and/or recipient EBV serostatus on transplant outcomes in patients with nonmalignant hematological disorders undergoing allo-HCT. A trend toward higher risk of cGVHD was observed in the case of EBV-seropositive donor, yet more data are necessary to draw a final conclusion.

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Authors' contributions

JS, LG, GT – study design. JS, GT, LG, SvdW, NSK, MM, PL – data analysis and interpretation. JS, GT, LG – manuscript writing. JS, LG, PL, DA, GS, HV, JHD, MA, AK, YB, AT, BA, BL, HLW, XP, JM, EP, PC, NM, JAS, IYA, JC, NS, CD, RPdL, AL, SC – provision of important clinical data. NSK, SvdW, GT – data check-up. GT – statistical analysis. JS, NSK, SC – administrative support. All authors – revision of manuscript, final approval.

Conflict of interest

None declared.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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