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ORIGINAL RESEARCH

Applying Rituximab During the Conditioning Regimen Prevents Epstein–Barr Virus Infection Following Allogeneic Hematopoietic Stem Cell Transplant in a Children's Cohort: A Retrospective Case–Control Study

Yongsheng Ruan · Libai Chen · Tingting Luo · Danfeng Xie · Wei Cao · Xuan Liu · Qiujun Liu · Yuhua Xiao · Cuiling Wu · Jianyun Wen · Juan Li · Jiangnan Meng · Xuedong Wu · Xiaoqin Feng

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ABSTRACT

Introduction: Since hematopoietic stem cell transplant (HSCT) is an important therapy for malignant and non-malignant pediatric diseases, improving transplant-related mortality remains a challenge. Currently, rituximab, a monoclonal antibody of anti-CD20, is widely used for several post-HSCT complications. However, few studies have focused on the application of rituximab before HSCT.

Methods: We conducted a retrospective case–control study from January 2019 to July 2021 to determine this effect in a single center. Forty-eight patients were included in the rituximab group, with a one-to-one ratio matched to the control group.

Results: Both the occurrence rate and cumulative incidence rate of Epstein–Barr virus (EBV) infection were significantly lower in the rituximab group than in the without-rituximab group (10.4% vs. 33.3%, $p = 0.014$ and 12.2% vs. 39.3% $p = 0.0026$, respectively). Furthermore, without the application of rituximab was identified as a risk factor for post-HSCT EBV infection via both univariate [hazard ratio (HR) = 4.17, 95%CI (1.52–11.43), $p = 0.005$] and multivariate analyses [HR = 4.65, 95%CI (1.66–13.0), $p = 0.003$]. Although the overall survival (OS) probability of the rituximab group was comparable to the without-rituximab group, a markedly improved OS of the rituximab group was found in the malignant disease subgroup (78.9% vs. 42.1%, $p = 0.032$). The outcomes of graft-versus-host disease, neutrophil and platelet engraftment, other viral infections, and the reconstitution of lymphocytes showed no significant differences between the two groups.

Conclusions: The administration of rituximab before HSCT may prevent EBV infection following HSCT.

Yongsheng Ruan, Libai Chen, Tingting Luo have contributed equally to this work.

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Y. Ruan · L. Chen · T. Luo · D. Xie · W. Cao · X. Liu · Q. Liu · Y. Xiao · C. Wu · J. Wen · J. Li · J. Meng · X. Wu (✉) · X. Feng (✉)
Department of Pediatrics, Nanfang Hospital, Southern Medical University, Guangzhou, China
e-mail: xuedongwu@163.com

X. Feng
e-mail: fxq126126@126.com

Keywords: Rituximab; B cell; Hematopoietic stem cell transplant; Epstein–Barr virus; Children

Key Summary Points

Less Epstein–Barr virus infection occurred post-hematopoietic stem cell transplant in the setting of applying rituximab during the conditioning regimen.

Among malignant disease patients, improved overall survival was indicated in the with-rituximab group.

No difference in graft-versus-host disease and recovery of both neutrophil and platelet was found between the with- or without-rituximab groups.

No delayed immune reconstitution was found in the applying rituximab group.

INTRODUCTION

Hematopoietic stem cell transplant (HSCT) is a potent therapy for malignant and non-malignant hematologic diseases in children [1, 2]. Both T- and B-cell lymphocytes play a critical role in the different stages of HSCT, including the mechanism of graft-versus-host disease (GVHD), promotion of engraftment, response to infection, and eradication of minimal residual leukemia [3–6]. Here, we summarize the B-cell-relevant complications of HSCT. First, B-cell lymphocytes play a crucial role in the production of autoantibodies. Hence, B cells are commonly involved in the mechanisms of autoimmune hemolytic anemia (AIHA), immune thrombocytopenic purpura (ITP) in both pre- and post-HSCT periods [7–9], and donor-specific antibodies (DSAs), which may lead to graft failure following HSCT [10]. Second, B cells also mediate the activation of T cells and induce the release of cytokines via antigen presentation. This effect is one of the potential mechanisms of transplant-associated thrombotic microangiopathy (TA-TMA) [11]. Third, this effect might lead to the promotion of acute GVHD (aGVHD). For chronic GVHD (cGVHD), B-cell activating factor (BAFF) seems to be important in the reconstitution and survival of B cells following HSCT, promoting the production of autoreactive B cells and the inhibition of regulatory T cells [8]. And fourth, memory B cells are the main host of the Epstein–Barr virus (EBV) [12]. EBV infection mainly results in EBV viremia, EBV diseases, EBV-associated post-transplant lymphoproliferative disease (PTLD), and lymphoma after HSCT [13]. This information indicates the critical role of B cells in the entire procedure of HSCT; therefore, depleting B cells with rituximab, a monoclonal antibody of anti-CD20, may represent a potentially feasible strategy for minimizing post-HSCT complications. To date, the use of rituximab as part of a conditioning regimen has not yet been thoroughly investigated. Therefore, we conducted a retrospective nested case–control study to address the effect of the strategy.

METHODS

Patients

All cases were enrolled from 232 children who underwent allogeneic HSCT in the Department of Pediatrics, Nanfang Hospital, Southern Medical University, China, from January 2019 to July 2021. Forty-eight cases who received rituximab as an agent in their conditioning regimen of HSCT were included in the with-rituximab group. For each with-rituximab case, one without-rituximab control case was randomly selected from the same cohort and was matched according to the following criteria: (1) age at the time of HSCT (± 5 years), (2) consistent diseases (± 1 case), (3) ratio of HLA-matched/mismatched types of HSCT (± 15%), (4) positive rate of direct antiglobulin test (Coomb's test) (± 15%), and (5) positive rate of platelet antibody test (solid-phase assay) (± 15%). Patients who had severe organ disorders, severe anemia (< 40 g/L) and thrombocytopenia (< 5×10^9/L), or positive DSA prior to

transplantation were excluded from this study. The median follow-up time was 3 years. All guardians of the subjects provided informed consent for their inclusion in the retrospective study. The study was conducted in accordance with the Helsinki Declaration of 1964 and its later amendments, and the protocol was approved by the Ethics Committee of Nanfang Hospital, Southern Medical University (NFEC-2022–522).

Conditioning Regimens and the Administration of Rituximab

The conditioning regimens were depicted in previous studies [14–17]. Briefly, the myeloablative conditioning regimens consisted of busulfan/cyclophosphamide/fludarabine with or without thiotepa. Post-transplant cyclophosphamide (PTCY) was applied on day + 3 and day + 4 for haploidentical HSCT patients. Anti-thymocyte globulin (ATG) was administrated for HLA-matched HSCT and thalassemia major (TM) haploidentical HSCT patients. Non-relative CB was applied on day+6 as a complementary graft source in haploidentical HSCT patients. Rituximab was administrated at a dose of 375 mg/m^2 per day on day–1 and day–8 within the conditioning regimen. The reduced-intensity conditioning (RIC) regimen comprised cyclophosphamide/fludarabine/thiotepa with PTCY.

Definitions

EBV infection included EBV DNAemia and end-organ disease. EBV DNAemia was measured in plasma using quantitative PCR (qPCR) (positive when > 100 IU/ml). EBV disease was defined as a positive EBV-encoded RNA (EBER) biopsy or positive EBV-DNA in bronchoalveolar lavage fluid or cerebral spinal fluid by qPCR with supporting clinical manifestation. Cytomegalovirus (CMV) infection was defined as the detection of > 500 IU/ml viral nucleic acid in plasma by qPCR [18]. PTLD and lymphoma were diagnosed using a biopsy in addition to positron emission tomography-computed tomography (PET-CT) [19]. Other viruses, including human herpesvirus 6, polyomaviruses, varicella-zoster virus, and herpes simplex virus 1 were detected via qPCR or metagenomic next-generation sequencing. Neutrophil recovery was defined as achieving an absolute neutrophil count of $\geq 0.5 \times 10^9$/L for 3 consecutive days; platelet recovery as platelets $\geq 20 \times 10^9$/L without transfusion for 7 days; and hemoglobin recovery as hemoglobin $\geq$ 70 g/L without transfusion for 7 days. Graft failure (GF) was indicated by an ANC of $< 0.5 \times 10^9$/L by day+30 with associated pancytopenia [20]. Poor graft function (PGF) was defined as persistent neutropenia (ANC $< 0.5 \times 10^9$/L), thrombocytopenia (platelets $< 20 \times 10^9$/L), and/or hemoglobin < 70 g/L for at least 3 consecutive days by day+28 with transfusion requirement in the presence of complete donor chimerism without disease relapse [20, 21]. Secondary failure of platelet recovery (SFPR) was defined as a platelet level of $< 20 \times 10^9$/L for 7 consecutive days or transfusion requirement after reaching a platelet level of $\geq 50 \times 10^9$/L without transfusion for 7 days post-HSCT [22]. Both acute and chronic GVHD were strictly diagnosed according to the published criteria [23, 24]. The diagnosis of TA-TMA referred to the criteria of Jodele et al. [25].

Statistic Analyses

Continuous variables were compared using *t* tests or the Kruskal–Wallis test. Categorical variables were compared via the χ^2 or Fisher's exact test. Hazard ratios (HR) for EBV infection post-HSCT were computed from univariate and multivariate Cox regression analyses. All the factors with $p < 0.25$ in the univariate analysis were included in the multivariate regression. Competing risk analysis was used to calculate the cumulative rates of GVHD and EBV infection. The probability of overall survival (OS) was determined using the Kaplan–Meier method, and the OS between the two groups was compared using the log-rank test. A p value of < 0.05 was considered statistically significant. All analyses were conducted in the R software (v.4.2.2, http://www.r-project.org).

RESULTS

Characteristics and Overall Outcome of Patients

Forty-eight patients were enrolled in the rituximab group, and the same number of patients were placed in the without-rituximab group. The baseline statistical information for the groups is shown in Table 1. The mean age was 7 years old for the entire population. The most common disease was TM, accounting for 39.6%, followed by acute leukemia, including acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), accounting for 22.9%. Other malignant diseases included juvenile myelomonocytic leukemia (JMML), chronic myeloid leukemia (CML), and hemophagocytic lymphohistiocytosis (HLH), while the non-malignant diseases comprised primary immunodeficiency disorders, such as Wiskott–Aldrich syndrome (WAS), chronic granulomatous disease (CGD), and severe combined immunodeficiency (SCID). Other malignancies accounted for 16.7%, while other non-malignancies accounted for 10.4%; severe aplastic anemia (SAA) accounted for 10.4%. More than half of the patients underwent HLA-mismatched HSCT. None of the major characteristics of HSCT were significantly different between the groups, apart from the pre-HSCT platelet antibodies ($p = 0.047$).

The primary outcomes of HSCT are summarized in Table 2. There was a statistically higher incidence of positive platelet antibodies following HSCT and a markedly lower incidence of EBV infection in the rituximab group than in the without-rituximab group ($p = 0.021$ and $p = 0.014$, respectively). The median times of neutrophil and platelet recovery were not statistically different between the rituximab group and the without-rituximab group (23 days and 30 days vs. 22 days and 17 days, $p = 0.895$ and $p = 0.186$, respectively). Furthermore, the incidences of GF, PGF, SFPR, and TA-TMA were quite similar between groups, showing no statistically significant differences.

The cumulative incidence rate (CIR) of grade III–IV aGVHD was $14.6\% \pm 5.1\%$ in the rituximab group and $27.1\% \pm 6.4\%$ in the without-rituximab group ($p = 0.15$) (Fig. S1A). Furthermore, according to Cox-model multivariate analysis (including all $p < 0.05$ factors from univariate analysis) of III–IV aGVHD, malignant disease ($p = 0.001$), mismatched donor ($p = 0.022$), PTCY + ATG prophylaxis (vs. PTCY alone, $p = 0.002$), TA-TMA ($p < 0.001$) were identified as risk factors (Supplementary Table S1). Regarding cGVHD, although the CIR of the rituximab group was lower than that of the without-rituximab group ($38.6\% \pm 7.3\%$ and $50.7\% \pm 7.9\%$, respectively), no statistically significant difference was found ($p = 0.26$) (Fig. S2A). In addition, no significant differences were found in aGVHD and cGVHD in the malignancy (Figs. S1B, S2B) and non-malignancy (Figs. S1C, S2C) subset analyses.

Overall Survival

The OS rate of the rituximab group was $83.3\% \pm 5.1\%$ [95%CI (73.3%–94.6%)], while the OS rate was $68.7\% \pm 6.7\%$ [95%CI (56.8–83.2%)] in the without-rituximab group ($p = 0.1$) (Fig. 1A). Furthermore, the OS of the rituximab group was significantly higher than in the without-rituximab group in the malignancy subset {$78.9\% \pm 9.4\%$ [95%CI (62.6–99.6%)] vs. $42.1\% \pm 1.1\%$ [95%CI (24.9–71.3%)], respectively, $p = 0.032$} (Fig. 1B). However, the same OS was observed in both the with and without-rituximab groups in the non-malignancy subset {$86.2\% \pm 6.4\%$ [95%CI (74.5–99.7%)], $p = 0.97$} (Fig. 1C). In the univariate Cox regression analysis, non-malignancy was a protective factor ($p = 0.006$), while a higher number of MNC ($p = 0.038$), TA-TMA ($p = 0.002$), and grade III–IV aGVHD ($p = 0.001$) were risk factors. In the multivariate analysis, non-malignancy was still a protective factor ($p = 0.023$), and only TA-TMA was identified as an independent risk factor ($p = 0.017$) (Supplementary Table S2). Severe pneumonia, aGVHD, TA-TMA, and relapse were the main causes of death (Supplementary Table S3).

Table 1 Characteristics of patients treated with and without rituximab

Characteristics	With rituximab $n = 48$	Without rituximab $n = 48$	p value
Age (years, mean (SD))	7.68 (4.01)	7.12 (3.93)	0.494
Sex (%)			
Male	32 (66.7)	33 (68.8)	1
Female	16 (33.3)	15 (31.2)	
Disease (%)			
TM	19 (39.6)	19 (39.6)	1
AL	11 (22.9)	11 (22.9)	
Other malignancy	8 (16.7)	8 (16.7)	
SAA	5 (10.4)	5 (10.4)	
Other non-malignancy	5 (10.4)	5 (10.4)	
Direct antiglobulin test pre-HSCT (%)			
Negative	16 (33.3)	20 (41.7)	0.15
Positive	26 (54.2)	17 (35.4)	
N/A	6 (12.5)	11 (22.9)	
Platelet antibody test pre-HSCT (%)			
Negative	21 (43.8)	21 (43.8)	0.047
Positive	16 (33.3)	7 (14.6)	
N/A	11 (22.9)	20 (41.7)	
Donor type			
Matched sibling donor	8 (16.7)	8 (16.7)	0.974
Matched unrelated donor	11 (22.9)	11 (22.9)	
Mismatched unrelated	3 (6.2)	2 (4.2)	
Haploidentical donor	26 (54.2)	27 (56.2)	
Application of CB (%)			
No CB	25 (52.1)	31 (64.6)	0.301
CB engraftment	23 (47.9)	17 (35.4)	
Conditioning regimen (%)			
MAC	47 (97.9)	47 (97.9)	1
RIC	1 (2.1)	1 (2.1)	
Prophylaxis of GVHD (%)			
PTCY + CNI + MMF	15 (31.2)	13 (27.1)	0.974
PTCy + ATG + CNI + MMF	19 (39.6)	22 (45.8)	

Table 1 continued

Characteristics	With rituximab $n = 48$	Without rituximab $n = 48$	p value
ATG + CNI + MMF	5 (10.4)	5 (10.4)	
ATG + CNI + MMF + MTX	9 (18.8)	8 (16.7)	
MNC ($\times 10^8$/kg, median [IQR])	20.10 [9.50, 27.65]	20.66 [8.75, 27.25]	0.515
CD34 ($\times 10^6$/kg, median [IQR])	8.95 [4.21, 13.07]	9.25 [5.06, 13.47]	0.602

AL acute leukemia, *ATG* antithymocyte globulin, *CB* cord blood, *CMV* cytomegalovirus, *CNI* calcineurin inhibitors, *HLA* human leukocyte antigens, *HSCT* hematopoietic stem cell transplant, *IQR* interquartile range, *MAC* myeloablative conditioning, *MMF* mycophenolate mofetil, *MNC* mononuclear cells, *MTX* methotrexate, *N/A* not available, *PTCY* post-transplantation cyclophosphamide, *RIC* reduced-intensity conditioning, *SAA* severe aplastic anemia, *SD* standard deviation, *TM* thalassemia major

EBV Infection

All donors and recipients tested negative for EBV DNA before HSCT. The CIR of EBV infection was dramatically lower in the rituximab group than in the without-rituximab group (12.2% ± 5.1% vs. 39.3% ± 8.1, $p = 0.0026$, respectively) (Fig. 2A). Similar results were found in the further subset analyses. In the context of the malignancy subset, the CIR of EBV infection was 13.1% ± 8.7% in the rituximab group and 53.7% ± 15.3% in the without-rituximab group, with a p value of 0.024 (Fig. 2B). Similarly, in the setting of non-malignant disease, the CIRs of EBV infection were 11.8% ± 6.4% and 32.7% ± 9.0% in the with and without-rituximab groups, respectively ($p = 0.037$) (Fig. 2C). There were no statistical differences in EBV infection between matched donors and mismatched/haploidentical donors (Fig. S3).

The features of EBV infection are depicted in Table 3. Only five patients suffered an EBV infection in the rituximab group, while 16 patients were infected in the without-rituximab group. Although the recurrence of EBV, organs involved by EBV, EBV-related PTLD, and EBV-related lymphoma were only found in the without-rituximab group, there were no statistically significant differences. In detail, among the six organ-involved patients, two were involved in the respiratory system, two in the gastrointestinal system, and two in the central nervous system. Of note, the median time of onset of EBV infection was later in the rituximab group than in the without-rituximab group, despite no statistically significant difference (430 days vs. 118 days, $p = 0.057$).

Univariate and multivariate analyses were then performed to determine the relevant factors (Table 4). The results showed that only the application of rituximab was identified as a significant protective factor in both univariate ($p = 0.005$) and multivariate analyses ($p = 0.003$).

Reconstitution of Lymphocytes

Although the reconstitution of B cells (CD3-CD19 +) was lower in the rituximab group, no statistically significant difference was found. The mean number of B cells (CD3-CD19 +) was 11.3/μl ± 25.1/μl in the rituximab group and 82.3/μl ± 155.2/μl in the without-rituximab group, with a p value of 0.101 at 3 months post-HSCT (Fig. 3A). In addition, 419.8/μl ± 404.6/μl B cells were found in the rituximab group at 12 months post-HSCT, while 395.7/μl ± 520.0/μl B cells were found in the without-rituximab group ($p = 0.868$) (Fig. 3B). There were no significant differences among the lymphocyte T cells (CD3 +), subtypes of T cells (CD3 + CD4 + CD8- and CD3 + CD4-CD8 +), and NK cells (CD3-CD16 + CD56 +), neither at 3 months nor 12 months post-HSCT (Fig. 3).

Table 2 Clinical outcomes of patients treated with and without rituximab

Events	With rituximab $n = 48$	Without rituximab $n = 48$	p value
Direct antiglobulin test post-HSCT (%)			
Negative	2 (4.2)	9 (18.8)	0.075
Positive	37 (77.1)	30 (62.5)	
N/A	9 (18.8)	9 (18.8)	
Platelet antibody test post-HSCT (%)			
Negative	21 (43.8)	15 (31.2)	0.021
Positive	15 (31.2)	8 (16.7)	
N/A	12 (25.0)	25 (52.1)	
Recovery of neutrophil (days, median [IQR])	23.00 [19.00, 28.50]	22.00 [20.00, 31.25]	0.895
Recovery of hemoglobin (days, median [IQR])	23.00 [15.75, 33.25]	20.00 [16.00, 27.25]	0.39
Recovery of platelet (days, median [IQR])	29.50 [14.00, 40.25]	17.00 [12.75, 37.50]	0.186
EBV infection (%)			
No	43 (89.6)	32 (66.7)	0.014
Yes	5 (10.4)	16 (33.3)	
CMV infection (%)			
No	24 (50.0)	25 (52.1)	1
Yes	24 (50.0)	23 (47.9)	
Virus infection other than EBV and CMV (%)			
No	37 (77.1)	32 (66.7)	0.364
Yes	11 (22.9)	16 (33.3)	
Combined virus infection ($\geq$ 2 types of viruses) (%)			
No	24 (50.0)	23 (47.9)	1
Yes	24 (50.0)	25 (52.1)	
Graft failure (%)			
No	47 (97.9)	44 (91.7)	0.358
Yes	1 (2.1)	4 (8.3)	
Poor graft function (%)			
No	22 (45.8)	27 (56.2)	0.414
Yes	26 (54.2)	21 (43.8)	
SFPR (%)			
No	45 (93.8)	42 (87.5)	0.484
Yes	3 (6.2)	6 (12.5)	

Table 2 continued

Events	With rituximab $n = 48$	Without rituximab $n = 48$	p value
TA-TMA (%)			
No	44 (91.7)	45 (93.8)	1
Yes	4 (8.3)	3 (6.2)	
CIR of Grade III–IV acute GVHD (%)	14.6 ± 5.1	27.1 ± 6.4	0.15
CIR of chronic GVHD (%)			0.26
+ 6 month	20.5 ± 6.1	35.2 ± 7.4	
+ 12 month	38.6 ± 7.3	50.7 ± 7.9	

CIR cumulative incidence rate, *CMV* cytomegalovirus, *EBV* Epstein–Barr virus, *GVHD* graft-versus-host disease, *HSCT* hematopoietic stem cell transplant, *IQR* interquartile range, *SFPR* secondary failure of platelet recovery, *TA-TMA* transplant-associated thrombotic microangiopathy

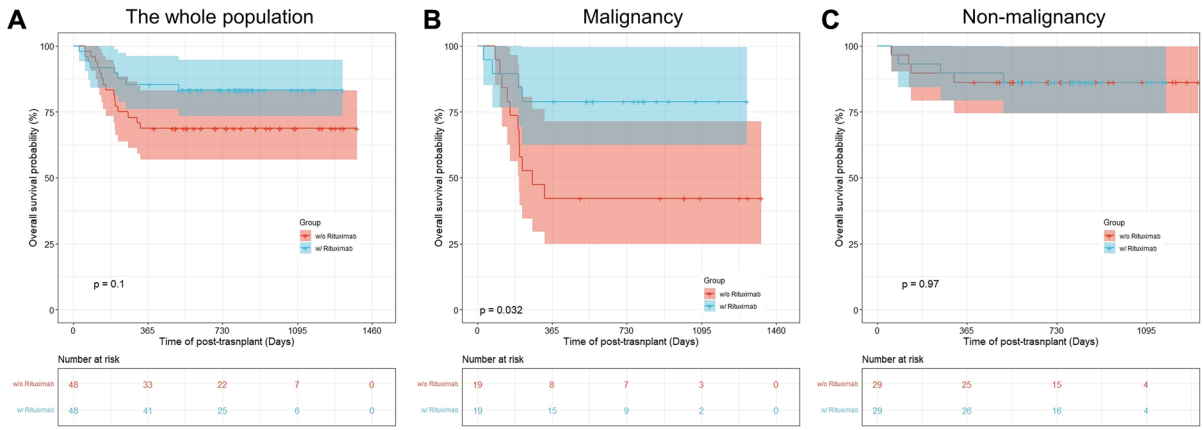

Fig. 1 Overall survival probability with and without-rituximab for: **A** the whole population; **B** the malignant disease subset; and **C** the non-malignant disease subset. The *red curve* shows the without (*w/o*)-rituximab group accompanied by a *red shadow* indicating a 95%CI, while the *blue curve* and *blue shadow* show the with (*w/*)-rituximab group

DISCUSSION

The present nested case–control study compared the survival outcomes and major complications reported with or without rituximab in the conditioning regimen in a children's cohort. To the best of our knowledge, our study is the first report of its kind in a pediatric cohort. Both multivariate analysis and the comparison of CIR showed that the occurrence of EBV infection was significantly lower in the rituximab group than in the without-rituximab group, despite having comparable OS probability. The subgroup of patients with malignant diseases had a higher OS probability in the rituximab group than in the without-rituximab group. Thus, our results indicate the possibility of exploring the administration of rituximab in patients with malignant diseases and a high risk of EBV infection.

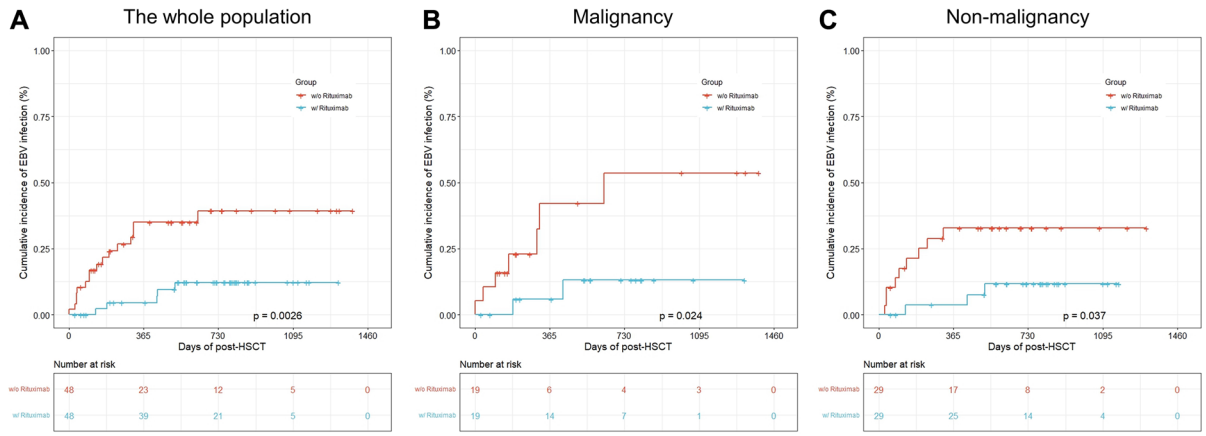

Fig. 2 Cumulative incidence of EBV infection in: **A** the entire population; **B** the malignant disease subgroup; and **C** the non-malignant disease subgroup. The *red curve* indicates the without (*w/o*)-rituximab group while the *blue curve* represents the with (*w/*)-rituximab group

Table 3 Features of EBV infection with and without rituximab

	With rituximab ($n = 5$)	**Without rituximab ($n = 16$)**	***p***
Recurrence of EBV (%)	0 (0.0)	4 (25.0)	0.555
Highest copies of EBV (median [IQR])	1010.00 [532.00, 1470.00]	4575.00 [672.25, 11,370.00]	0.355
Median copies of EBV (median [IQR])	624.00 [147.00, 1016.00]	2310.00 [608.00, 3730.00]	0.257
Duration of EBV infection (days, median [IQR])	21.00 [10.00, 80.00]	13.50 [8.25, 29.75]	0.404
Organs involved by EBV infection (%)	0 (0.0)	6 (37.5)	0.292
PTLD (%)	0 (0.0)	4 (25.0)	0.555
Lymphoma (%)	0 (0.0)	1 (6.2)	1
Onset of EBV infection (days, median [IQR])	430.00 [186.00, 432.00]	117.50 [39.50, 252.25]	0.057
Combined with CMV (%)	2 (40.0)	6 (37.5)	1

CMV cytomegalovirus, *EBV* Epstein–Barr virus, *IQR* interquartile range, *PTLD* post-transplant lymphoproliferative disorders

Overall, the two groups were comparable according to their age, sex, diseases, direct antiglobulin test, platelet antibody test, HLA disparity and donor type, cord blood engraftment, prophylaxis of GVHD, conditioning regimen, and number of transfused stem cells (Table 1). Only the platelet antibody test was statistically different between the two groups, although all patients with platelet antibodies were enrolled in the without-rituximab group. The reason for this was that, originally, rituximab was designed for patients with autoantibodies, indicated by a positive direct antiglobulin test and platelet antibody test. As a result, there was no significant difference in the post-HSCT direct antiglobulin test between the groups, while a higher rate of positive platelet antibody tests post-HSCT was observed with rituximab (Table 2). According to our literature review, the incidence of post-HSCT AIHA is

Table 4 Univariate and multivariate analyses for EBV infection

Factors	Univariable HR (95% CI, *p* value)	Multivariable HR (95%CI, *p* value)
Age	0.93 (0.83–1.05, 0.227)	0.88 (0.77–1.01, 0.069)
Sex		
Male	Reference	Reference
Female	0.47 (0.16–1.39, 0.171)	0.53 (0.17–1.61, 0.300)
Disease		
Malignancy	Reference	
Non-malignancy	0.76 (0.32–1.81, 0.535)	
Donor		
Matched sibling donor	Reference	
Matched unrelative donor	0.81 (0.23–2.79, 0.736)	
Mismatched unrelative	0.63 (0.07–5.37, 0.670)	
Haploidentical donor	0.64 (0.22–1.88, 0.420)	
CB		
No CB	Reference	
CB engraftment	0.82 (0.34–1.99, 0.665)	
Prophylaxis of GVHD		
PTCY + CNI + MMF	Reference	Reference
PTCy + ATG + CNI + MMF	0.91 (0.29–2.86, 0.869)	0.8 (0.25–2.60, 0.700)
ATG + CNI + MMF	1.63 (0.39–6.84, 0.504)	1.18 (0.16–8.93, 0.900)
ATG + CNI + MMF + MTX	2.13 (0.65–6.99, 0.212)	2.64 (0.40–17.5, 0.300)
Rituximab		
With rituximab	Reference	Reference
Without rituximab	4.17 (1.52–11.43, 0.005)	4.65 (1.66–13.0, 0.003)
MNC	0.97 (0.92–1.01, 0.134)	0.98 (0.91–1.06, 0.600)
CD34 +	1.01 (0.95–1.07, 0.688)	
Neutrophil recovery	0.98 (0.93—1.03, 0.338)	
Platelet recovery	0.99 (0.96—1.01, 0.309)	
CMV		
No CMV	Reference	
CMV	0.67 (0.28–1.62, 0.372)	
aGVHD		
No aGVHD	Reference	

Table 4 continued

Factors	Univariable HR (95% CI, *p* value)	Multivariable HR (95%CI, *p* value)
Grade I–II aGVHD	1.05 (0.39–2.8, 0.924)	
Grade III–IV aGVHD	0.78 (0.22–2.76, 0.696)	
cGVHD		
No cGVHD	Reference	
cGVHD	1.25 (0.53–2.96, 0.604)	
PGF		
No PGF	Reference	
PGF	0.63 (0.26–1.52, 0.301)	

ATG antithymocyte globulin, *CB* cord blood, *CI* confidence interval, *CMV* cytomegalovirus, *CNI* calcineurin inhibitors, *GVHD* graft-versus-host disease, *HLA* human leukocyte antigens, *HR* hazard ratio, *MAC* myeloablative conditioning, *MMF* mycophenolate mofetil, *MNC* mononuclear cells, *MTX* methotrexate, *PGF* poor graft function, *PTCY* post-transplantation cyclophosphamide, *RIC* reduced-intensity conditioning

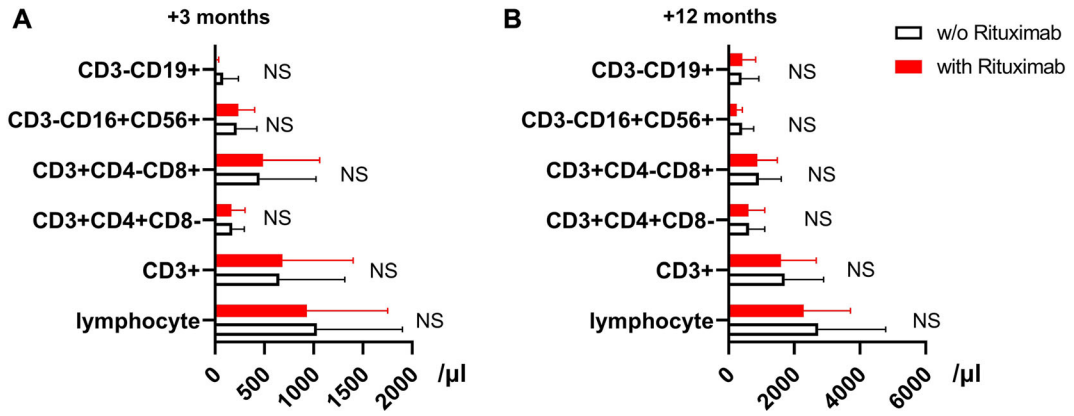

Fig. 3 Analyses of subgroups of lymphocytes at month+3 (**A**) and month+12 (**B**) following HSCT. The *red bars* show with the rituximab group while the *blank bars* show the without (*w/o*)-rituximab group (mean ± SD). *NS* non-significant

about 5%, whereas it can reach up to 20% in children with non-malignant diseases [7]. Moreover, the direct antiglobulin test may be positive in the absence of hemolysis resulting from other autoimmune conditions, intravenous immunoglobulin (IVIG), ATG, and daratumumab [26]. On the other hand, platelet antibodies were found to be one of the main causes of thrombocytopenia following HSCT [27]. Rituximab was recommended as an effective agent for the treatment of post-HSCT AIHA and ITP [28]. Both post-HSCT AIHA and ITP may result in poor survival outcomes [7, 27, 29]. The results of our study indicate that the application of rituximab in the conditioning regimen may not help with decreasing the occurrence of both hemoglobin and platelet antibodies. Furthermore, the engraftment of neutrophils and platelets and the occurrence of TA-TMA, PGF, and SFPR were quite comparable between the two groups in the present study (Table 2). These results are consistent with a

recent study that also focused on the administration of rituximab before HSCT [30, 31]. Since rituximab has been a recommended treatment for DSA in several studies [32–35], the DSA-positive patients were excluded from the present study.

Several studies have demonstrated that B cells contribute to acute GVHD, which may be abrogated by rituximab as part of a myeloablative or nonmyeloablative conditioning regimen in malignant diseases [30]. Patel et al. [31] stated that rituximab-based conditioning regimens did not reduce the incidence of aGVHD. Additionally, according to an animal study, host B cells may confer a protective effect on the initiation of aGVHD via the secretion of IL-10 [39]. Importantly, rituximab administered before transplantation appears to be safe [30, 40]. In our study, regarding aGVHD and cGVHD, there were no marked differences with or without the administration of rituximab before HSCT (Figs. S1, S2). Apart from lymphoma patients, incorporating rituximab into the conditioning regimen in HSCT has not been well investigated with respect to survival outcomes. According to a recent retrospective study, no differences in prognosis were observed between rituximab and non-rituximab groups, including non-relapse mortality, leukemia-free survival, and OS in adult patients undergoing allo-HSCT [30]. These results were similar to our study except for the malignant disease subgroup (Fig. 1), although there were the same number of acute leukemia and other malignant diseases between groups. Nonetheless, it may not be appropriate to draw conclusions due to the relatively small sample size used in this study.

Of note, we consistently found that rituximab administrated in the conditioning regimen prevented the incidence of EBV infection (Fig. 2). Although the serostatus of EBV was not available in our center, the seroprevalence of EBV stabilizes at over 90% after age 8 years in China [41]. Therefore, most EBV infections in the study were considered as reactivation rather than de novo infections. Similarly, two recent studies demonstrated that rituximab applied before HSCT in adult patients led to the elimination of EBV reactivation and EBV-related PTLD [30, 31]. The underlying mechanisms remain largely unclear. Typically, EBV can directly drive the proliferation of B cells, which are the primary targets and hosts of EBV [42]. Progressive EBV-associated PTLD or lymphoma are dismal outcomes of EBV infection [43, 44]. Adequate evidence has been found to support rituximab as a potent agent for the treatment of post-HSCT EBV infection in both children and adults [45–47]. Critically, it was a worthwhile strategy that closely monitored EBV reactivation and preemptive therapy using rituximab, especially for the patients at high risk of EBV-lymphoproliferative disease [48, 49]. Furthermore, serial studies have found that the main risk factors for EBV infection include high cumulative levels of immunosuppression, older age at transplantation, profound T-cell depletion, and the administration of ATG or alemtuzumab [13, 43, 44, 50]. Interestingly, PTCY, a strategy for T-cell depletion, was not used in the treatment of EBV infection, especially PTLD [51, 52]. In another recent study, no EBV reactivation was found in PTCY-based haploidentical HSCT in children with TM [53]. The potential reasons for this include the destruction of EBV-infected B cells, the allowance of a considerable dose of stem cells accompanying memory T cells, and rapid T-cell immune reconstitution [54, 55]. Regarding GVHD prophylaxis, about 25% of patients underwent ATG alone while about 40% of patients experienced ATG plus PTCY, and therefore the hazardous effect of ATG may be compensated by the protective effect of PTCY in the setting of combination in the current study (Tables 1, 4). Moreover, a reduction in immunosuppression, rituximab, or cellular immunotherapy are well-established methods in the management of EBV infection [13]. All patients who underwent EBV infection in the current study were treated as above. Although the post-HSCT administration of rituximab has been reported to be relatively safe, Launspach et al. demonstrated that the post-HSCT administration of rituximab caused prolonged B-cell impairment and increased the risk of infections in a children's cohort [56]. Unlike applying rituximab in the post-HSCT period [6, 56], we found that using rituximab prior to HSCT had little effect on delayed B cell immune reconstitution (Fig. 3). This still

requires further prospective studies for verification.

This study has certain limitations, including the general weaknesses of retrospective studies, the relatively small scale of the population, and the various conditioning regimens. Further prospective large cohort studies are required to confirm the results presented here.

CONCLUSIONS

We have evaluated the clinical outcomes in the setting of applying rituximab in the conditioning regimen. Crucially, the incidence of EBV reactivation was effectively decreased in the with-rituximab group. Moreover, the with-rituximab regimen may improve OS in malignant diseases. However, there was no significant effect on the prophylaxis of GVHD and the inhibition of the production of autoimmune antibodies, along with no delayed immune reconstitution.

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Disclosures. All named authors confirm that they have no conflicts of interest to declare.

Compliance with Ethics Guidelines. The study was conducted in accordance with the Helsinki Declaration of 1964 and its later amendments, and the protocol was approved by the Ethics Committee of Nanfang Hospital, Southern Medical University (NFEC-2022–522).

Data Availability. All data generated or analyzed during this study are included in this published article/as supplementary information files.

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