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Lower Absolute Lymphocyte Count Before Conditioning Predicts High Relapse Risk in Patients After Haploidentical Peripheral Blood Stem Cell Transplantation With Low Dose Anti-Thymocyte Globulin/Post-Transplant Cyclophosphamide for GvHD Prophylaxis

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Abstract

Standard anti-thymocyte globulin (ATG) weight-based dosing often resulted in highly variable ATG exposure, which had profound effects on relapse and survival, especially in recipients with relatively low absolute lymphocyte count (ALC) before conditioning. Data regarding rabbit ATG pharmacokinetics and pharmacodynamics in the setting of HLA-haploidentical peripheral blood stem cell transplantation (haplo-PBSCT) is lacking. We conducted a retrospective study on 90 consecutive patients who underwent haplo-PBSCT with low dose rabbit ATG (5 mg/kg) plus low dose post-transplant cyclophosphamide (50 mg/kg) based regimen for graft-versus-host disease (GvHD) prophylaxis. We compared serum concentration of ATG and post-transplant results between patients with ALC$<$500/μl and ALC$\geq$500/μl before conditioning. Patients with ALC$<$500/μl had higher ATG concentrations, delayed immune reconstitution, lower incidence of grade II-IV acute GvHD (0 *vs.* 19.42%, $P = 0.043$), higher risk of Epstein-Barr virus infection within 100 days post-transplant (47.78% *vs.* 22.22%, $P = 0.020$) and 1-year relapse rate (33.33% *vs.*11.59%, $P = 0.041$), and lower 1-year overall survival (OS) (52.38% *vs.*79.71%, $P = 0.004$), 1-year relapse free survival (RFS) (47.62% *vs.* 75.36% for RFS, $P = 0.014$), and 1-year GvHD free relapse-free survival (GRFS) (42.89% *vs.* 65.22%, $P = 0.043$). ALC$<$500/μl before conditioning was a significant poor risk factor for relapse, OS, RFS, and GRFS.

Keywords

absolute lymphocyte count, relapse, survival, haploidentical, peripheral blood stem cell transplantation, anti-thymocyte globulin

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Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an effective method for the treatment of malignant hematologic diseases. HLA-haploidentical peripheral blood stem cell transplantation (haplo-PBSCT) is more and more widely used. Disease relapse is the main cause of death after transplantation. There are many factors for disease relapse after transplantation, such as disease status before transplantation, conditioning regimen, and donor sources, and so on[1,2]. Anti-human thymocyte immunoglobin (ATG) dosage based on body weight has been used widely for many years[3]. Recent studies showed that body weight-based dosing of ATG often resulted in highly variable ATG exposures, which had profound effects on relapse and survival especially in recipients with low peripheral blood absolute lymphocyte counts (ALCs) before conditioning[4–7]. A higher serum-level of rabbit ATG at early time post-transplant seemed to be a strong predictor for relapse and poor survival in patients with HLA-matched allo-HSCT[8]. Data regarding rabbit ATG pharmacokinetics and pharmacodynamics in the setting of haplo-PBSCT is sparse because available literature had substantive variability in total and active ATG concentrations[9]. We hypothesized that lower ALCs before conditioning might be closely related to higher ATG concentration and higher risk of relapse in patients undergoing haplo-PBSCT with low dose rabbit ATG (5 mg/kg) and low dose post-transplant cyclophosphamide (50 mg/kg) (low dose ATG/PTCy) based regimen for graft-versus-host disease (GvHD) prophylaxis.

Methods

Patients, Donors, and Stem Cell Sources

We conducted a retrospective study on recipients of haplo-PBSCT from September 2017 to December 2019 in our center. All patients were diagnosed with hematologic malignancies. Family members selected as donors were typed on the HLA-A, -B, -C, -DRB1, and -DQB1 locus at high-resolution level with recipient-donor HLA mismatches$\geq$3 as haplotype[10]. All patients received peripheral blood stem cells (PBSCs) mobilized with granulocyte colony-stimulating factor. The target number of $CD34^+$cells in mobilized PBSC graft was a minimum of 8×10^6/kg. Some patients also received third-party 4/6~6/6 loci matched umbilical cord blood (UCB). The study was approved by the ethical committees of Shanghai General Hospital and conducted in accordance with the Declaration of Helsinki. All data originated from clinical trials was approved by patients with mandatory written informed consent.

Conditioning Regimens and GvHD Prophylaxis

Reduced-intensity conditioning was given to patients with acute myelocytic leukemia (AML) and myelodysplastic syndrome (MDS) aged 55 and above ($\geq$55 years), while myeloablative conditioning was given to those aged under 55 years old (<55 years)[11–13]. Myeloablative conditioning regimens were given to patients with non-Hodgkin's lymphoma (NHL) and acute lymphoblastic leukemia (ALL)[13]. All patients received low dose ATG/PTCy based regimen for GvHD prophylaxis[11–13], which included rabbit anti-thymocyte globulin (Thymoglobin®, Genzyme Polyclonals S.A.S) 2.5 mg/kg on day-2 and day-1, cyclophosphamide 50 mg/kg on day+3 (low dose ATG/PTCy) followed by cyclosporine A 2 mg/kg/d intravenously from day+4 and mycophenolate mofetil orally 720 mg three times per day from day+4 to day+34.

ALC and ATG Concentrations

Peripheral blood ALC was detected one day before conditioning. Blood serum for ATG concentrations test was collected and stored at -80°C from day -1 to day +27 after transplantation (d-1, d0, d+2, d+4, d+6, d+9, d+12, d+15, d+18, d+21, d+24, and d+27). ATG concentrations were tested with enzyme-linked immunosorbent assay (ELISA) by using a spectrophotometer (ATG ELISA Kit, You Xuan Biology, China).

CMV-DNA and EBV-DNA Detection

Quantitative real-time PCR assays for cytomegalovirus (CMV) DNA and Epstein-Barr virus (EBV) DNA in peripheral blood were performed weekly (Sino-American Biotech, Beijing, China). The cut-off value of CMV-DNA and EBV-DNA was set as 1000 copies/ml according to the manufacturer.

Definitions

The engraftment of neutrophil and platelet and graft failure were defined according to the literature[14]. The chimerism analysis was performed with polymerase chain reaction (PCR) amplification of short tandem repeats on CD3+ T lymphocytes of bone marrow[15], or fluorescent in situ hybridization for patients with sex-mismatched donors[16] every month within the first year post-transplant. Acute GvHD (aGvHD) and chronic GvHD (cGvHD) were diagnosed and graded according to the modified Glucksberg criteria[17] and the 2014 National Institutes of Health consensus criteria[18], respectively. Relapse was defined by European Society for Blood and Marrow Transplantation criteria[19].

Statistical Analysis

SPSS 25.0 and GraphPad Prism 5 were used for data analysis and drawing. Baseline characteristics were summarized using descriptive statistics. Fisher exact and chi-square tests

were used to compare categorical variables and the Wilcoxon rank-sum test was used to compare continuous variables. The cumulative incidences of GvHD, relapse and non-relapse mortality (NRM) were calculated and compared using Fine-Gray test. Overall survival (OS), relapse free survival (RFS) and GvHD-free relapse-free survival (GRFS) were estimated by the method of Kaplan–Meier and compared by the log-rank test. The prognostic factors for GvHD, relapse, and NRM were analyzed by the proportional subdistribution hazards regression model in competing risks and for OS, RFS, and GRFS were examined in Cox proportional hazards models. Factors with P value <0.200 in univariate analysis were included into multivariate analysis. ATG concentrations were compared by analysis of variance for repeated measurement data. A 2-sided P value <0.050 indicated statistical significance.

Results

Patient Characteristics

A total of 90 consecutive recipients of haploidentical transplantation in our center enrolled the study, which included 52 patients with AML, 18 patients with ALL, 13 patients with MDS, and 7 patients with NHL. The median age was 40 (7–64) years old. Twenty-one patients had ALC<500/μl before conditioning, while 69 patients had ALC≥500/μl. There were no significant differences in the clinical characteristics between ALC<500/μl and ALC≥500/μl groups. The details of patients' characteristics were shown in Table 1.

ATG Concentrations

Ten consecutive recipients of haplo-PBSCT from October 2019 to December 2019 had ATG concentrations test in the study, which included 4 patients with ALC<500/μl and 6 patients with ALC ≥500/μl. ATG concentrations in peripheral blood serum were measured at 12 timepoints from the day-1 to day +27 after transplantation. The ATG concentrations were significantly higher in ALC <500/μl group than that in ALC≥500/μl group at the same timepoint (d0: 108.90 *vs.* 67.43, $P = 0.004$; d+9: 72.61 *vs.* 47.84, $P = 0.019$; d+18: 56.40 *vs.* 40.37, $P = 0.052$; d+27: 41.27 *vs.* 30.80, $P = 0.090$; ug/ml) (Fig. 1).

Engraftment

The median mononuclear cells and CD34+ cells in the PBSC grafts were 16.8 (3.7~33)$\times10^8$/kg and 10.0 (2.8~29.5)$\times10^6$/kg respectively. Fifty-six patients received third-party UCB with the median numbers of nucleated cells 2.2 (0.6~4.2) $\times10^7$/kg and CD34+ cells 5.1 (1.1~12.2) $\times10^4$/kg, respectively. Two patients in ALC<500/μl group died of heart failure on day +5 and +9 after transplantation, respectively. Eighty-eight patients successfully engrafted and there was no primary graft failure. The median time of neutrophil and platelet engraftment were 13(10~21) and 15 (12~26) days, respectively. There were no differences in the time of neutrophil ($P = 1.000$) and platelet engraftment between the two groups ($P = 1.000$). All of the 88 patients achieved full donor chimerism on day+28 after transplantation.

Immune Reconstitution

The counts of CD3+, CD3+CD4+CD8-, CD3+CD4-CD8+, CD19+ and CD16+CD56+ (natural killer, NK) cells were higher in ALC ≥500/μl group than those in ALC<500/μl group at 3,6, 9 and 12 months after transplantation, although there were no significant differences. The data of immune reconstitution post-transplant of patients between the two groups were displayed in Supplementary Fig. 1.

GvHD

The median onset time of aGvHD was 21 (11~95) day after transplantation. The cumulative incidences of grade II-IV and III-IV aGvHD were 12.22 and 5.56% within 100 days, respectively. The cumulative incidences of grade I-IV and II-IV aGvHD were significantly lower in ALC <500/μl group than that in ALC≥500/μl group (14.28% *vs.* 40.58%, $P = 0.009$; 0 *vs.* 19.42%, $P = 0.043$) (Fig. 2A, B). No patients developed III-IV aGvHD in ALC <500/μl group, while 9.25% of patients in ALC≥500/μl group developed III-IV aGvHD ($P = 0.171$) (Fig. 2C). Univariate analysis for grade II-IV aGvHD was showed in Supplementary Table 1 and multivariate analysis showed that ALC≥500/μl before conditioning was an independent risk factor for grade II-IV aGvHD ($RR = 0.371$, $P = 0.048$) (Table 2).

The median onset time of cGvHD was 184 (123~952) days post-transplant. The 1-year cumulative incidences of all grades cGvHD and moderate to severe cGvHD were 31.11% and 16.67%, while the 3-year cumulative incidences were 33.33% and 17.78%, respectively. No significant difference between the two groups was noted in the cumulative incidence of moderate to severe cGvHD (10.53% vs. 20.00% for ALC<500/μl vs. ALC≥500/μl, $P = 0.318$) (Fig. 2D). Univariate analysis for grade moderate to severe cGvHD was showed in Supplementary Table 1. Donor age (≥40 years) might be an independent risk factor for moderate to severe cGvHD by multivariate analysis ($P = 0.058$) (Table 2).

CMV and EBV Infection

The cumulative incidence of CMV reactivation within 100 days post-transplant was 30.00% and two patients (2.22%) developed CMV disease. There was no significant difference

Table 1. Characteristics of Patients.

Variables	All patients (N = 90)	ALC<500/μl (N = 21)	ALC≥500/μl (N = 69)	*P* value
Recipient age, year, n (%)				0.879
<55	74 (82.22)	18 (85.71)	56 (81.16)	
≥55	16 (17.78)	3 (14.29)	13 (18.84)	
Recipient sex, n (%)				0.583
Male	56 (62.22)	12 (57.14)	44 (63.77)	
Female	34 (37.78)	9 (42.86)	25 (36.23)	
Diagnosis, n (%)				0.643
Myeloid	65 (72.22)	16 (76.19)	49 (71.01)	
Lymphoid	25 (27.78)	5 (23.81)	20 (28.98)	
Number of chemotherapy, median (range)	3 (0~13)	3 (0~10)	3 (0~13)	0.992
R-DRI, n (%)				0.557
Low	7 (7.78)	2 (9.52)	5 (7.24)	
Intermediate	68 (75.55)	14 (66.67)	54 (78.27)	
High	15 (16.67)	5 (23.81)	10 (14.49)	
Very high	0	0	0	
Disease status, n (%)				0.234
CR	61 (67.78)	12 (57.14)	49 (71.01)	
NR	29 (32.22)	9 (42.86)	20 (28.98)	
HCT-CI, n (%)				0.233
< 3	89 (98.89)	20 (95.24)	69 (100.00)	
≥3	1 (1.11)	1 (4.76)	0	
Donor age, yr, n (%)				0.173
< 40	62 (68.89)	17 (80.95)	45 (65.22)	
≥40	28 (31.11)	4 (19.95)	24 (34.78)	
Donor-recipient sex match, n (%)				0.766
Female to male	17 (18.89)	3 (14.29)	14 (20.29)	
Others	73 (81.11)	18 (85.71)	55 (79.71)	
Donor sources, n (%)				0.117
Parents	27 (30.00)	3 (14.29)	24 (34.78)	
Sibling	11 (12.22)	4 (19.04)	7 (10.15)	
Offspring	49 (54.45)	14 (66.67)	35 (50.72)	
Cousin	3 (3.33)	0	3 (4.35)	
ABO blood type, n (%)				0.291
Compatible	51 (56.67)	14 (66.67)	37 (53.62)	
Incompatible	39 (43.33)	7 (33.33)	32 (46.38)	
Conditioning regimen, n (%)				0.580
MAC	51 (56.67)	13 (61.91)	38 (55.07)	
RIC	39 (43.33)	8 (11.59)	31 (44.93)	
PBSC graft, median (range)				
Mononuclear cells, $x10^8$/kg	16.8 (3.7~33.0)	15 (3.7~31.0)	17 (4.3~33.0)	0.065
$CD3^+$cells, $x10^8$/kg	2.6 (2.2~6.0)	2.2 (0.8~4.3)	2.7 (0.7~6.0)	0.098
$CD34^+$cells, $x10^6$/kg	10.0 (2.8~29.5)	12.9 (2.8~22.7)	10.1 (2.8~29.4)	0.057
UCB, n (%)				0.320
Yes	56 (62.22)	15 (71.43)	41 (59.42)	
No	34 (37.78)	6 (28.57)	28 (40.58)	
UCB, median (range)				
NCs, $x10^7$/kg	2.2 (0.6~4.2)	2.0 (0.8~3.3)	2.5 (0.6~4.2)	0.078
$CD34^+$cells, $x10^4$/kg	5.1 (1.1~12.2)	4.1(1.1~10.7)	5.6 (1.2~12.2)	0.083

ALC: absolute lymphocyte count; R-DRI: refined disease risk index; CR: complete remission; NR: non-remission; HCT-CI: hematopoietic cell transplantation comorbidity index; PBSC: peripheric blood stem cell; MAC: myeloablative conditioning; RIC: reduced-intensity conditioning; UCB: umbilical cord blood; NC: nucleated cell.

in the cumulative incidences of CMV reactivation between the 2 groups within 100 days after transplantation (33.33% *vs.*25.56% for ALC<500/μl vs. ALC≥500/μl, $P = 0.926$).

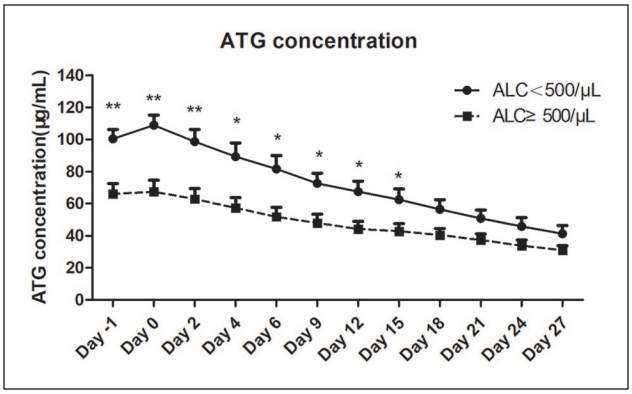

Figure 1. Serum concentrations of anti-thymocyte globulin (ATG) in ALC <500/μl group and ALC≥500/μl group from day -1 to day +27 posttransplant. (Data were shown as mean ATG concentration, $P_{d-1} = 0.006$; $P_{d0} = 0.004$; $P_{d+2} = 0.008$; $P_{d+4} = 0.016$; $P_{d+6} = 0.017$; $P_{d+9} = 0.020$; $P_{d+12} = 0.018$; $P_{d+15} = 0.038$; $P_{d+18} = 0.052$; $P_{d+21} = 0.066$; $P_{d+24} = 0.086$; $P_{d+27} = 0.090$). ALC: absolute lymphocyte count.

However, the mean CMV viral load by PCR was significantly higher in ALC <500/μl group than that in ALC≥500/μl group (2 852 025 *vs.*90 701 copies/ml, $P = 0.001$) (Fig. 3A). The cumulative incidence of EBV reactivation within 100 days was 28.89% and only one two patients (1.11%) developed post-transplant lymphoproliferative disorders. The cumulative incidence of EBV reactivation within 100 days and mean EBV viral load after transplantation were significantly higher in ALC <500/μl group than that in ALC≥500/μl group, respectively (47.78% *vs*. 22.22%, $P = 0.020$ for EBV reactivation; 275 005 *vs.* 52 824 copies/mL for EBV-DNA load, $P = 0.001$) (Fig. 3B).

Relapse and NRM

A total of 17 (18.89%) patients relapsed, the median time to relapse were 229 (75~875) days. The 1-year cumulative incidence of relapse was significantly higher in ALC <500/μl group than that in ALC ≥500/μl group (33.33% *vs.*11.59%, $P = 0.041$) (Fig. 4A). Univariate analysis for relapse was shown in Supplementary Table 2. ALC <500/μl was the only risk factor for relapse in multivariable analysis (*RR*=2.713, $P = 0.047$) (Table 2).

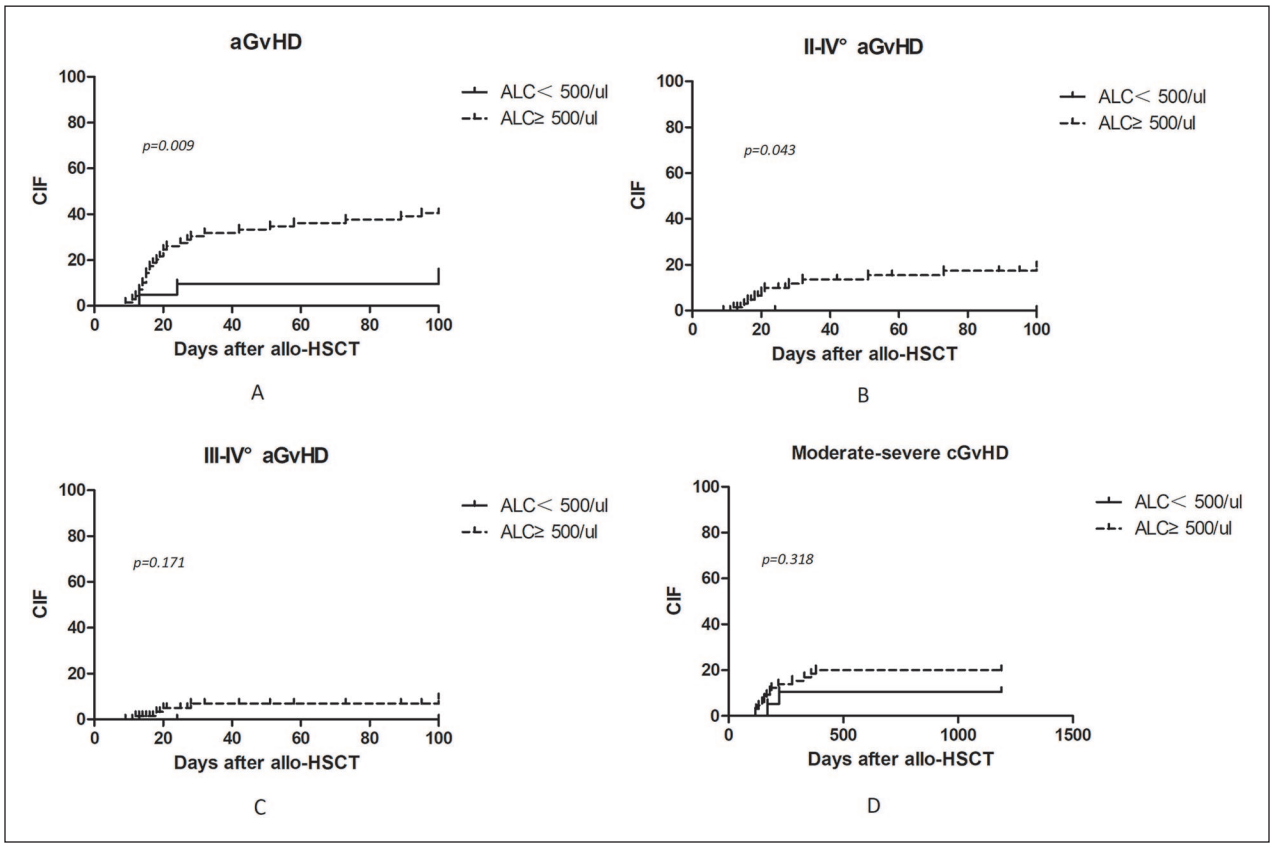

Figure 2. The cumulative incidences function (CIF) of graft versus host disease (GvHD) in ALC <500/μl group and ALC≥500/μl group. (A) The CIF of grade I-IV acute GvHD (aGvHD). (B) The CIF of grade II-IV aGvHD. (C) The CIF of grade III-IV aGvHD. (D) The CIF of grade moderate-severe chronic GvHD (cGvHD). aGvHD: acute graft versus host disease; ALC: absolute lymphocyte count; cGvHD: chronic graft versus host disease; HSCT: hematopoietic stem cell transplantation.

Table 2. Multivariate Analysis for GvHD, Relapse and Survival.

	Factors	RR	95% CI	*P* value
II-IV°aGvHD	Recipient sex (Female vs. Male)	0.149	0.019~1.168	0.072
	ABO blood type (incompatible vs. compatible)	2.718	0.776~9.520	0.118
	Conditioning regimen (MAC vs. RIC)	1.441	0.409~5.083	0.570
	ALC (<500/μl vs. ≥500/μl)	0.371	0.115~1.200	0.048
Moderate to severe cGvHD	Donor age (<40 vs.≥40, year)	0.265	0.673~1.041	0.058
	ABO blood type (incompatible vs. compatible)	2.674	0.935~7.644	0.067
	Disease status (CR vs. NR)	0.546	0.145~2.060	0.372
CIR	Donor age (<40 vs.≥40, yr)	0.408	0.117~1.429	0.161
	Umbilical cord bloodCD34$^+$cells (10^5/kg)	0.594	0.164~2.147	0.427
	ALC (<500/μl vs. ≥500/μl)	2.713	0.983~7.484	0.047
NRM	Recipient age (≥55 vs.<55, yr)	3.935	1.398~11.074	0.009
	Conditioning regimen (MAC vs. RIC)	2.974	0.798~11.080	0.104
OS	Disease status (CR vs. NR)	0.724	0.297`1.763	0.476
	Umbilical cord blood CD34$^+$cells (10^4/kg)	1.578	0.681~3.655	0.287
	ALC (<500/μl vs. ≥500/μl)	4.090	1.900~8.800	0.022
RFS	Disease status (CR vs. NR)	0.613	0.255~1.473	0.274
	ALC (<500/μl vs. ≥500/μl)	4.180	2.040~8.595	0.018
GRFS	Umbilical cord blood (Yes vs. No)	0.539	0.250~1.162	0.115
	ALC (<500/μl vs. ≥500/μl)	1.966	1.008~3.837	0.047

GvHD: chronic graft-versus-host disease; RR: relative ratio; CI: confidence interval; aGvHD: acute graft-versus-host disease; MAC: myeloablative conditioning; RIC: reduced-intensity conditioning; ALC: absolute lymphocyte count; cGvHD: chronic graft-versus-host disease; CR: complete remission; NR: non-remission; CIR: cumulative incidence of relapse; NRM: non-relapse mortality; OS: overall survival; RFS: relapse free survival; GRFS: graft-relapse-free survival.

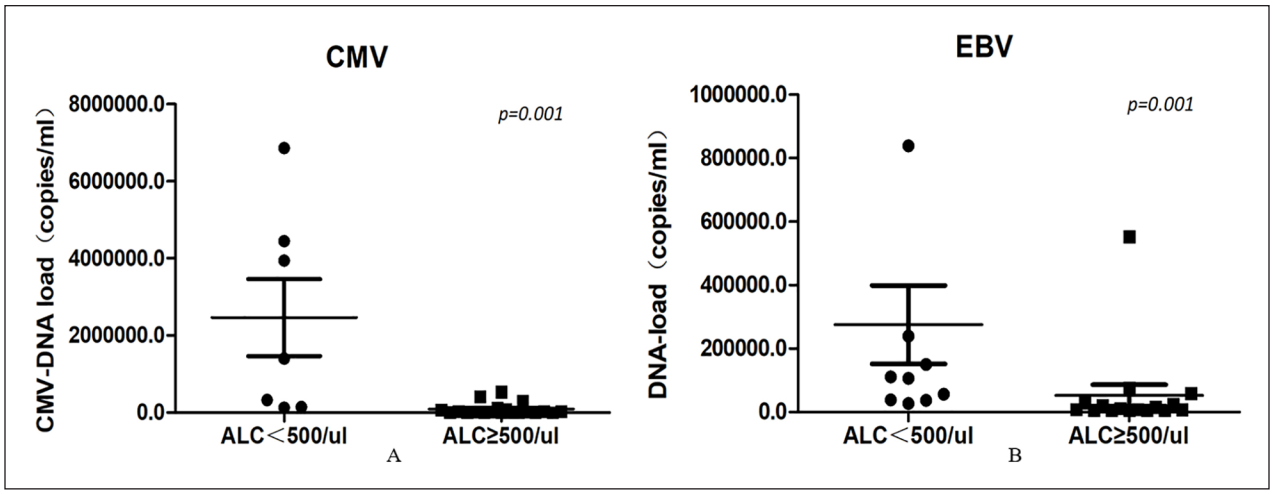

Figure 3. CMV and EBV viral loads within 100 days after transplantation in ALC <500/μl group and ALC≥500/μl group. (A) CMV viral load. (B) EBV viral load. ALC: absolute lymphocyte count; CMV: cytomegalovirus; EBV: Epstein-Barr virus; DNA: deoxyribonucleic acid.

Fifteen patients died of non-relapse causes (16.67%), 9 of them died of infection, 2 of heart failure, 2 of graft rejection, 1 of cerebral hemorrhage and 1 of grade IV aGvHD. The 1-year NRM in ALC <500/μl group was similar with that in ALC ≥500/μl group (19.05% *vs.*13.00%, $P = 0.722$) (Fig. 4B). Univariate analysis for NRM was shown in Supplementary Table 2. Recipient aged≥55 years old was the only independent risk factor for NRM in multivariable analysis ($RR = 3.935$, $P = 0.009$) (Table 2).

OS, RFS and GRFS

After a median follow-up of 540 (12~1213) days,12 patients (57.14%) in ALC <500/μl group and 17 patients (24.64%) in ALC ≥500/μl group died. The 1-year OS and RFS were significantly lower in ALC <500/μl group than that in ALC ≥500/μl group (52.38% *vs.*79.71% for OS, $P = 0.004$; 47.62% *vs.* 75.36% for RFS, $P = 0.014$) (Fig. 5A, B). The 1-year GRFS in ALC <500/μl group was also significantly

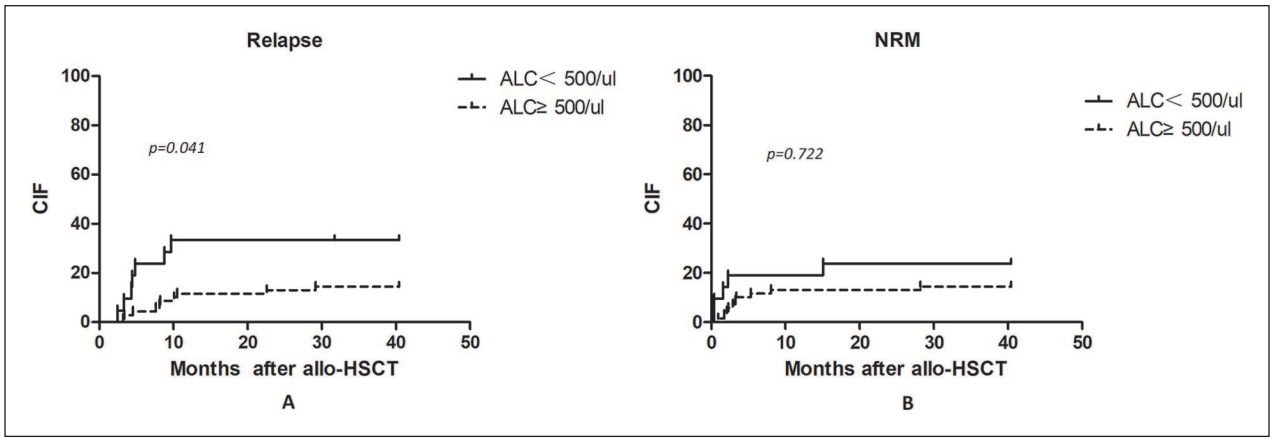

Figure 4. The cumulative incidences function (CIF) of relapse and non-relapse mortality (NRM) in ALC <500/μl group and ALC≥500/μl group. (A) The CIF of relapse. (B) The CIF of NRM. ALC: absolute lymphocyte count; HSCT: hematopoietic stem cell transplantation.

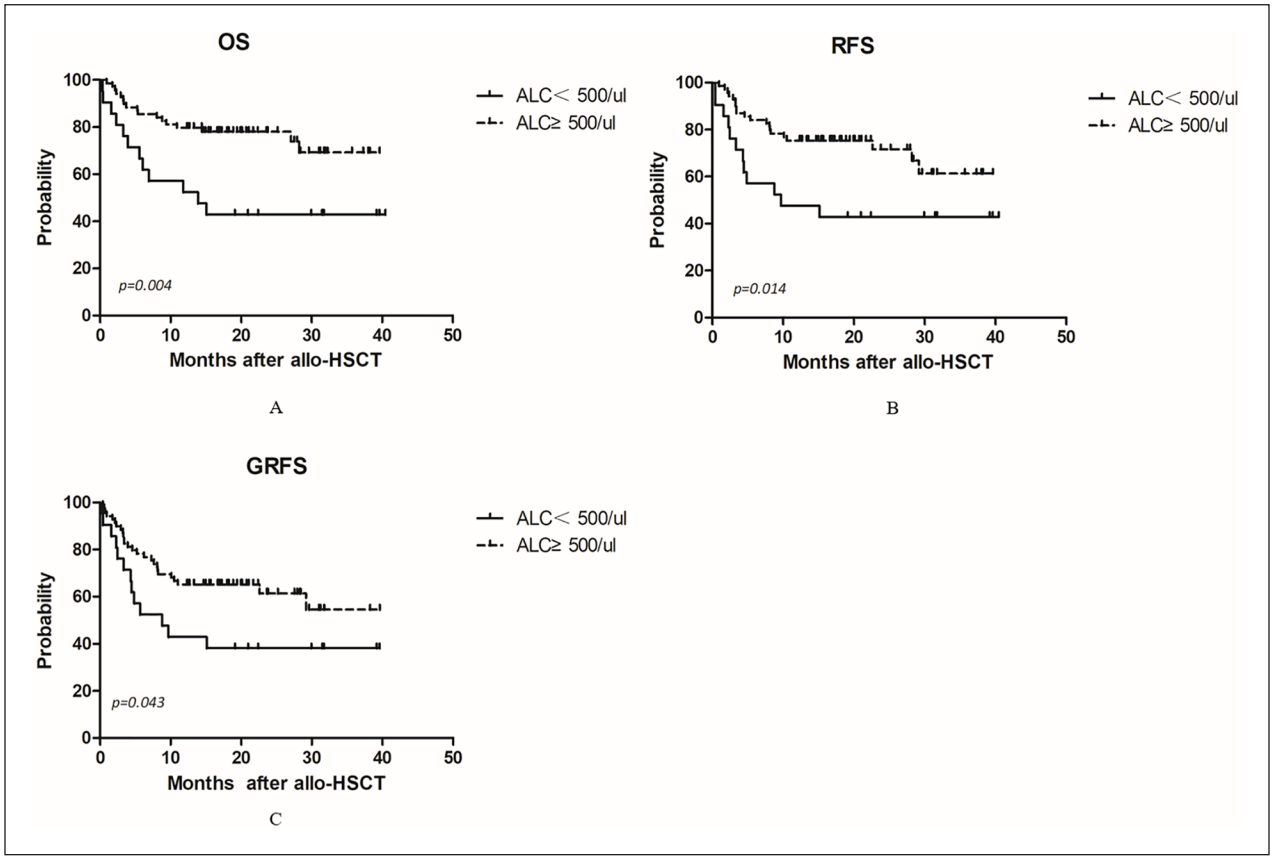

Figure 5. The probabilities of overall survival (OS), relapse-free survival (RFS) and GvHD-free, relapse-free survival (GRFS) in ALC <500/μl group and ALC≥500/μl group. (A) The probability of OS. (B) The probability of RFS. (C) The probability of GRFS. ALC: absolute lymphocyte count; GvHD: graft-versus-host disease; HSCT: hematopoietic stem cell transplantation.

lower than that in ALC ≥500/μl group (42.89% *vs.* 65.22%, $P = 0.043$) (Fig. 5C). Univariate analysis for OS, RFS, and GRFS was shown in Supplementary Table 3. Multivariable analysis showed that ALC<500/μl was the only significant risk factor for OS ($RR = 4.090$, $P = 0.022$), RFS ($RR = 4.180$, $P = 0.018$), and GRFS ($RR = 1.966$, $P = 0.047$) (Table 2).

Discussion

The present study demonstrated that ALC before conditioning was an important prognostic factor for patients undergoing haplo-PBSCT with low dose ATG/PTCy based regimen for GvHD prophylaxis. The patients with ALC<500/μl before conditioning had higher blood concentration of ATG, lower incidence of GvHD, higher incidence of relapse, and lower 1-year OS and RFS, than patients with ALC≥500/μl.

First, we analyzed potential clinical factors on ALC before conditioning. Our data showed there were no significant differences in age, diagnosis, R-DRI, disease status, HCT-CI and numbers of chemotherapy between ALC<500/μl group and ALC≥500/μl group.

Remberger et al. and Lindemans et al. found that the pharmacokinetics of ATG was influenced by recipient's age, ALC before conditioning, the number of infused donor cells, anti-ATG antibodies and individual bio-degradation function[8,20]. In our study, there were no statistical differences in recipient ages and PBSC graft (numbers of MNC/CD3+/CD34+ cells) between the two groups, therefore ALC before conditioning might be an important factor associated with ATG clearance. It was reported that for patients with lower ALCs before conditioning, higher dosage of ATG resulted in more unbinding form of ATG in circulation[21]. Excessive ATG continued to deplete passenger lymphocytes of the donor graft, even subsequent repopulation of the T cells from donor hemopoietic stem cell[22]. It might take up to almost 2 years to reconstitute a diverse, self-tolerant and naive T-cell repertoire after allo-HSCT[22]. Furthermore, ATG destroys not only T lymphocytes in the recirculating pool but also B lymphocytes, NK cells, and even dendritic cells, which led to severe non-specific immunosuppression and delayed immune reconstitution[23,24]. Our study showed patients with lower ALC before conditioning had significantly lower counts of $CD4^+$and NK cells counts at 3 months after transplantation and delayed immune reconstitution.

Our study showed the CIs of grade I-IV and II-IV aGvHD were significantly lower in ALC <500/μl group than those in ALC ≥500/μl group. A retrospective study on recipients of allo-HSCT from matched related donor showed the CIs of grade I-IV (14.3% *vs.* 44.4%, $P = 0.040$) and II-IV(7.1% *vs.*31.5%, $P = 0.048$) aGvHD were significantly lower in ALC <500/μl group as compared with ALC ≥500/μl group[25]. The lower ALC leads to a higher ATG concentration and subsequently redundant ATG could eliminate more alloreactive donor T lymphocytes that mediate GvHD[26].

The lower ALC before conditioning was associated with higher risk of CMV and EBV viral infections in our study. A recent study on 135 patients who received HLA matched unrelated PBSCT with 3 different dosages of ATG reported that higher ATG dosages were associated with increased risk of infections. There was no difference in ALC among the three groups[27]. Hannon et al. found that higher ATG concentration on day 28 post-transplant was associated with CMV infection[28]. Relative excessive ATG could do harm to immune reconstitution, which could lead to reduction of antiviral T lymphocytes and lack of diversity of T cell receptor[29]. In the present study, the immune reconstitution was delayed in patients with ALC<500/μl as compared with patients with ALC ≥500/μl.

The 1-year relapse rate was significantly higher in ALC <500/μl group than that in ALC ≥500/μl group in our study. Remberger et al. reported a similar result that patients with higher ATG concentrations had higher risk of relapse than those with lower ATG concentrations (82% *vs.* 17%, $P < 0.010$)[8]. A multicenter retrospective study found excessive exposure to ATG resulted in higher risk of relapse with substantially lower ALC[7]. For recipients with lower ALC before conditioning, the graft versus leukemia effect might be attenuated, which would increase the risk of relapse.

In terms of survival, our study showed ALC<500/μl was related to lower 1-year OS, RFS and GRFS. ALC<500/μl was the only significant risk factor for OS, RFS, and GRFS, but not for NRM. A study on matched related donor allo-HSCT with rabbit ATG (4.5 mg/kg) showed that the 1-year NRM was significantly higher in ALC <500/μl group as compared with ALC ≥500/μl group (28.6% vs. 8.6%; $P = 0.031$)[25]. The study also showed that the median survival in ALC <500/μl group was significantly inferior to that in ALC≥ 500/μl group (291, 217.7 to 364.3 days vs. OS not reached; $P = 0.001$)[25]. Podgorny et al. reported that higher ATG concentration was a risk factor for infectious complications and increased mortality, which might increase the NRM, mostly via an infectious complication[8,30]. However, in the present study, there was no significant difference in NRM between the two groups, the reason might be the different GvHD prophylaxis. Our previous study showed the low dose ATG/PTCY produced lower incidence of infection compared with classic GvHD prophylaxis with full dose of ATG[11].

Nevertheless, this study had some limitations. First, it was a retrospective analysis with limited number of patients from a single institution. Second, the study included patients with different hematological malignancies. Third, only a small part of patients had ATG concentration testing.

In conclusion, our study demonstrated that for patients undergoing haplo-PBSCT with low dose ATG/PTCy based regimen for GvHD prophylaxis, ALC <500/μl before conditioning was related to higher ATG concentration, lower risk of GvHD, but higher risk of relapse and poor survival. Weight based ATG dosing would lead to over exposure in patients with low ALC. ATG dosage adjustment on ALC before conditioning might be a more reasonable and precise strategy, which needs further prospective studies.

Ethical Approval

This study was approved by Clinical Research Ethics Committee of Shanghai General Hospital, China.

Statement of Human and Animal Rights

All procedures in this study were conducted in accordance with the Shanghai Municipal Clinical Research Ethics Committee approved protocols.

Statement of Informed Consent

Written informed consent was obtained from the patients for their anonymized information to be published in this article.

Declaration of Conflicting Interests

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Supplemental Material

Supplemental material for this article is available online.

References

1. Wang Y, Chen H, Chen J, Han M, Hu J, Jiong H, Huang H, Lai Y, Liu D, Liu Q, Liu T, et al. The consensus on the monitoring, treatment, and prevention of leukemia relapse after allogeneic hematopoietic stem cell transplantation in China. Cancer Lett. 2018;438:63–75.
2. Zhan H. Leukemia relapse after transplantation—a consensus on monitoring, prevention, and treatment in China. BMC Med. 2019;17(1):34.
3. Jullien M, Guillaume T, Peterlin P, Garnier A, Le Bourgeois A, Debord C, Mahe B, Dubruille V, Wuilleme S, Blin N, Touzeau C, et al. Antithymocyte globulin administration in patients with profound lymphopenia receiving a PBSC purine analog/busulfan-based conditioning regimen allograft. Sci Rep. 2020;10(1):15399.
4. Admiraal R, van Kesteren C, Jol-van der Zijde CM, van Tol MJ, Bartelink IH, Bredius RG, Boelens JJ, Knibbe CA. Population pharmacokinetic modeling of Thymoglobulin(®) in children receiving allogeneic-hematopoietic cell transplantation: towards improved survival through individualized dosing. Clin Pharmacokinet. 2015;54(4):435–46.
5. Admiraal R, Lindemans CA, van Kesteren C, Bierings MB, Versluijs AB, Nierkens S, Boelens JJ. Excellent T-cell reconstitution and survival depend on low ATG exposure after pediatric cord blood transplantation. Blood. 2016;128(23):2734–41.
6. de Koning C, Admiraal R, Nierkens S, Boelens JJ. Immune reconstitution and outcomes after conditioning with antithymocyte-globulin in unrelated cord blood transplantation; the good, the bad, and the ugly. Stem Cell Investig. 2017;4:38.
7. Admiraal R, Nierkens S, de Witte MA, Petersen EJ, Fleurke GJ, Verrest L, Belitser SV, Bredius RGM, Raymakers RAP, Knibbe CAJ, Minnema MC, et al. Association between antithymocyte globulin exposure and survival outcomes in adult unrelated haemopoietic cell transplantation: a multicentre, retrospective, pharmacodynamic cohort analysis. Lancet Haematol. 2017;4(4):e183–91.
8. Remberger M, Persson M, Mattsson J, Gustafsson B, Uhlin M. Effects of different serum-levels of ATG after unrelated donor umbilical cord blood transplantation. Transpl Immunol. 2012;27(1):59–62.
9. McCune JS, Bemer MJ, Long-Boyle J. Pharmacokinetics, pharmacodynamics, and pharmacogenomics of immunosuppressants in allogeneic hematopoietic cell transplantation: part II. Clin Pharmacokinet. 2016;55(5):551–93.
10. O'Donnell PV, Luznik L, Jones RJ, Vogelsang GB, Leffell MS, Phelps M, Rhubart P, Cowan K, Piantados S, Fuchs EJ. Nonmyeloablative bone marrow transplantation from partially HLA-mismatched related donors using posttransplantation cyclophosphamide. Biol Blood Marrow Transplant. 2002;8(7):377–86.
11. Xu X, Yang J, Cai Y, Li S, Niu J, Zhou K, Jiang Y, Xu X, Shen C, Huang C, Qiu H, et al. Low dose anti-thymocyte globulin with low dose posttransplant cyclophosphamide (low dose ATG/PTCy) can reduce the risk of graft-versus-host disease as compared with standard-dose anti-thymocyte globulin in haploidentical peripheral hematopoietic stem cell transplantation combined with unrelated cord blood. Bone Marrow Transplant. 2021;56(3):705–708.
12. Yang J, Jiang J, Cai Y, Li S, Wan L, Zhu J, Liu H, Shao S, Bai H, Wang C, Song X. Low-dose anti-thymocyte globulin plus low-dose posttransplant cyclophosphamide as graft-versus-host disease prophylaxis in haploidentical peripheral blood stem cell transplantation combined with unrelated cord blood for patients with hematologic malignancies: a prospective, phase II study. Bone Marrow Transplant. 2019;54(7):1049–57.
13. Sun X, Yang J, Cai Y, Wan L, Huang C, Qiu H, Tong Y, Xu X, Zhou K, Ding X, Song X. Low-dose antithymocyte globulin plus low-dose posttransplant cyclophosphamide combined with cyclosporine and mycophenolate mofetil for prevention of graft-versus-host disease after HLA-matched unrelated donor peripheral blood stem cell transplantation. Bone Marrow Transplant. 2021;56:2423–31.
14. Martinelli G, Trabetti E, Farabegoli P, Testoni N, Bandini G, Motta MR, Vittone A, Terragna C, Pignatti PF, Tura S. Early detection of bone marrow engraftment by amplification of hypervariable DNA regions. Haematologica. 1997;82(2):156–60.
15. Willasch AM, Kreyenberg H, Shayegi N, Rettinger E, Meyer V, Zabel M, Lang P, Kremens B, Meisel R, Strahm B, Rossig C, et al. Monitoring of hematopoietic chimerism

after transplantation for pediatric myelodysplastic syndrome: real-time or conventional short tandem repeat PCR in peripheral blood or bone marrow? Biol Blood Marrow Transplant. 2014;20(12):1918–25.
16. Wang Y, Wu DP, Liu QF, Xu LP, Liu KY, Zhang XH, Yu WJ, Xu Y, Huang F, Huang XJ. Low-dose post-transplant cyclophosphamide and anti-thymocyte globulin as an effective strategy for GVHD prevention in haploidentical patients. J Hematol Oncol. 2019;12(1):88.
17. Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA, Lerner KG, Thomas ED. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation. 1974;18(4):295–304.
18. Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW, Palmer J, Weisdorf D, Treister NS, Cheng GS, Kerr H, et al. 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. 2015;21(3): 389–401.e1.
19. Forghieri F, Comoli P, Marasca R, Potenza L, Luppi M. Minimal/measurable residual disease monitoring in NPM1-mutated acute myeloid leukemia: a clinical viewpoint and perspectives. Int J Mol Sci. 2018;19(11):3492.
20. Lindemans CA, Chiesa R, Amrolia PJ, Rao K, Nikolajeva O, de Wildt A, Gerhardt CE, Gilmour KC, Bierings M, Veys P, Boelens JJ, et al. Impact of thymoglobulin prior to pediatric unrelated umbilical cord blood transplantation on immune reconstitution and clinical outcome. Blood. 2014;123(1):126–32.
21. Modi D, Kim S, Surapaneni M, Ayash L, Ratanatharathorn V, Uberti JP, Deol A. Absolute lymphocyte count on the first day of thymoglobulin predicts relapse-free survival in matched unrelated peripheral blood stem cell transplantation. Leuk Lymphoma. 2020;61(13):3137–45.
22. Simons L, Cavazzana M, André I. Concise review: boosting T-cell reconstitution following allogeneic transplantation-current concepts and future perspectives. Stem Cells Transl Med. 2019;8(7):650–57.
23. Nishihori T, Al-Kadhimi Z, Hamadani M, Kharfan-Dabaja MA. Antithymocyte globulin in allogeneic hematopoietic cell transplantation: benefits and limitations. Immunotherapy. 2016;8(4):435–47.
24. Ito A, Kitano S, Tajima K, Kim Y, Tanaka T, Inamoto Y, Kim SW, Yamamoto N, Fukuda T, Okamoto S. Impact of low-dose anti-thymocyte globulin on immune reconstitution after allogeneic hematopoietic cell transplantation. Int J Hematol. 2020;111(1):120–30.
25. Woo GU, Hong J, Kim H, Byun JM, Koh Y, Shin DY, Kim I, Yoon SS. Preconditioning absolute lymphocyte count and transplantation outcomes in matched related donor allogeneic hematopoietic stem cell transplantation recipients with reduced-intensity conditioning and antithymocyte globulin treatment. Biol Blood Marrow Transplant. 2020;26(10):1855–60.
26. Vo PT, Pantin J, Ramos C, Cook L, Cho E, Kurlander R, Khuu H, Barrett J, Leitman S, Childs RW. Conditioning with rabbit versus horse ATG dramatically alters clinical outcomes in identical twins with severe aplastic anemia transplanted with the same allogeneic donor. J Hematol Oncol. 2015;8:78.
27. Kennedy VE, Chen H, Savani BN, Greer J, Kassim AA, Engelhardt BG, Goodman S, Sengsayadeth S, Chinratanalab W, Jagasia M. Optimizing antithymocyte globulin dosing for unrelated donor allogeneic hematopoietic cell transplantation based on recipient absolute lymphocyte count. Biol Blood Marrow Transplant. 2018;24(1):150–55.
28. Hannon M, Beguin Y, Ehx G, Servais S, Seidel L, Graux C, Maertens J, Kerre T, Daulne C, de Bock M, Fillet M, et al. Immune recovery after allogeneic hematopoietic stem cell transplantation following flu-TBI versus TLI-ATG conditioning. Clin Cancer Res. 2015;21(14):3131–39.
29. Soares MV, Azevedo RI, Ferreira IA, Bucar S, Ribeiro AC, Vieira A, Pereira PNG, Ribeiro RM, Ligeiro D, Alho AC, Soares AS, et al. Naive and stem cell memory T cell subset recovery reveals opposing reconstitution patterns in CD4 and CD8 T cells in chronic graft vs. host disease. Front Immunol. 2019;10:334.
30. Podgorny PJ, Ugarte-Torres A, Liu Y, Williamson TS, Russell JA, Storek J. High rabbit-antihuman thymocyte globulin levels are associated with low likelihood of graft-vs-host disease and high likelihood of posttransplant lymphoproliferative disorder. Biol Blood Marrow Transplant. 2010;16(7):915–26.