High proportions of CD3+ T cells in grafts delayed lymphocyte recovery and reduced overall survival in haploidentical peripheral blood stem cell transplantation

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Received April 26, 2019; Accepted February 2, 2020

DOI: 10.3892/mco.2020.2027

Abstract. T cells in grafts serve an important role in the pathogenesis of graft versus host disease (GVHD) and immune recovery during HLA matched allogeneic stem cell transplantation. However, the role of T cells in the haploidentical peripheral blood stem cell transplantation (Haplo-PBSCT) is yet to be determined. In the present study, the role of CD3+ T cells in grafts and impact on hematopoietic and immune recovery, cytomegalovirus (CMV) reactivation, GVHD, relapse, progress free survival and overall survival (OS) were evaluated and analyzed. A total of 30 patients who underwent haplo-PBSCT were included in the present study. CD3+ T cells accounted for a median of 23.1% (range 8-47.4%) with a median dose of 299.7x10^6/kg (range 104-623.4). Patients were divided into two groups according to the CD3+ T cell count: Above the median (high T cell group) and below the median CD3+ T cell (low T cell group). No significant difference was identified between neutrophil and platelet recovery time between two groups (P>0.05). The mean lymphocyte recovery time of high T cell group and low T cell group were 107.07 days (95% CI 79.88-134.25), and 50.4 days (95% CI 41.42-59.38), respectively. The lymphocyte recovery time of high T cell group was higher that of low T cell group (P<0.05). No significant difference between CMV reactivation, chronic GVHD and primary disease relapse rates was observed between two groups (P>0.05). The cumulative incidence of grade II or above acute GVHD was higher in the high T groups compared with low T groups (P<0.05). The overall survival and progress free survival rates were higher in the low T cell group compared with the high T cell group (P<0.05). In conclusion, high levels of CD3+ T cells in the grafts were associated with delayed lymphocyte recovery and an increased risk of acute GVHD and decreased overall survival.

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Key words: haploidentical peripheral blood stem cell transplantation, lymphocyte recovery, CD3 positive T cells, graft versus host disease

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative therapy for malignant and non-malignant hematologic disorders. Haploidentical stem cell transplantation is a treatment option for patients who do not have an HLA-matched sibling or unrelated donor. The disadvantages of haploidentical HSCT are high incidence of graft-versus-host disease (GVHD), graft rejection, and delayed or incomplete immune reconstitution. Currently, the source of stem cells includes bone marrow, granulocyte colony-stimulating factor mobilized peripheral blood stem cells and umbilical cord blood. Different compositions of the grafts have different effects on the hematopoietic and immune recovery, GVHD and overall survival (OS) (1). Mature donor CD3+ T cells in the graft will recognize MHC molecules or peptides on the surface of host cells and induce acute GVHD. However, depletion of T cells in the graft results in graft failure, prolonged immunosuppression and leukemia relapse, thus innovative strategies are needed to limit the GVHD related pathological effects of donor alloreactive CD3+ T cells while maintaining their graft versus leukemia (GVL) effects. Impact of CD3+ T cells in the grafts on the clinical outcomes in different type of HSCT is still uncertain. It was reported that the incidence of the acute GVHD was higher in the graft with high counts of CD3+ T cells in HLA-matched HSCT, but it was also reported that high counts of CD3+ T cells resulted in more intensive GVL without producing more severe GVHD and resulted in better OS in haploidentical bone marrow combined with peripheral stem cells transplantation (2,3). To date, there was rare report about the impacts of CD3+ T cells in grafts on the hematopoietic recovery, GVHD

and OS in haplo-PBSCT. In the present study, we investigate the correlations between the $CD3^+$ T cells in the graft with hematopoietic recovery, GVHD, disease relapse, progress free survival (PFS) and OS in haplo-PBSCT.

Patients and methods

The study included 30 patients who underwent haploidentical HSCT between January 2015 and December 2017 at The First Affiliated Hospital of Xi'an Jiaotong University (Xi'an, China). Only acute leukemia or myelodysplastic syndrome patients were eligible for the study. Patients and donors were 5/10 HLA-mismatched using high-resolution typing at HLA-A/B/C/DRB1/ DQB1. All patients underwent haplo-PBSCT with the modified BuCy2 myeloablative conditioning regimen. All patients received ATG 2.5 mg/kg for continuous 4 days for GVHD prophylaxis, and cyclosporin, short-term methotrexate and mycophenolate mofetil were also included for GVHD prophylaxis.

This study was approved by the Ethics Comittee of the First Affiliated Hospital of Xi'an Jiaotong University. All of patients and donors, or their legal guardians, provided written informed consent in accordance with the Declaration of Helsiniki.

Mobilization and stem cell collection. For donor stem cell mobilization, 10 μg/kg/day G-CSF for consecutive four days was given to healthy donors by subcutaneous injection and then stem cell collection was carried out.

Graft content. The number of total nucleated, $CD34^+$ and $CD3^+$ cells in the donor graft were assessed before stem cell infusion. $CD34^+$ and $CD3^+$ cells were calculated by flow cytometer, data were acquired and analyzed by Flowjo 10 software (Tree Star Inc., Ashland, OR, USA).

Hematopoietic engraftment. Neutrophil, platelet and lymphocyte engraftment were defined as the first three consecutive days with an absolute count of >0.5, >20, >1x10^9/l respectively.

GVHD diagnosis and treatment. Acute and chronic GVHD was diagnosed according to the Seattle criteria (4). All patients with grade II or above acute GVHD were treated with 1 to 2 mg/kg/day methylprednisolone and chronic GVHD was initial treated with 1 mg/kg/day prednisone.

Cytomegalovirus (CMV) infection and CMV reactivation prophylaxis. Prophylaxis of CMV infection was instructed as previous study and CMV reactivation was defined as detection of CMV-DNA positive twice by PCR in serum.

Relapse, PFS and OS. Relapse was recorded as disease recurrence. Progress free survival (PFS) was from the day of transplantation to the disease relapse. Overall survival (OS) was from the disease onset to death or last follow-up.

Statistical analysis. Chi-square test was used to compare categorical variables and the Mann-Whitney rank-sum test was used to compare the absolute cell counts. Hematopoietic recovery, GVHD, CMV reactivation and relapse were assessed using cumulative incidence with competing risk. OS and PFS were obtained using Kaplan-Meier and compared by log-rank test. Multivariate analysis was performed using the Logistic hazards. $P<0.05$ was considered statistically significant. All analyses were performed using SPSS 13.0 (SPSS, Inc.) software.

Results

Graft content. The median mononuclear cells and $CD34^+$ cells transplanted was 10.9x10^8/kg (range 8.04-15.19) and 7.2x10^6/kg (range 2.14-17.43) respectively. $CD3^+$ T cells accounted for a median of 23.1% (range 8-47.4%) with a median dose of 299.7x10^6/kg (range 104-623.4). There was no significant difference of $CD3^+$ T cells proportion and counts between the first and the second day harvest ($P>0.05$). There was no significant difference of $CD3^+$ T cell in the grafts between donors with different sex and age ($P>0.05$). To test whether the $CD3^+$ T cell count in donor graft was associated with the hematopoietic reconstitution, GVHD, PFS and OS. The recipients were divided into two groups according to the $CD3^+$ T cell count: Above the median (high T cell group) and below the median $CD3^+$ T cell (low T cell group). The baseline characteristics of two groups were comparable (Table I).

Neutrophil, platelet and lymphocyte engraftment. The mean time to neutrophil engraftment was 10.13 days (95% CI 9.22-11.04) in high T cell group, and was 9.13 days (95% CI 8.55-9.72) in low T cell group. The mean time to platelet engraftment was 33.27 days (95% CI 5.41-61.12) in high T cell group, and was 11.53 days (95% CI 9.59-13.47) in low T cell group. The mean time to lymphocyte recovery was 107.07 days (95% CI 79.88-134.25) in high T cell group, and was 50.4 days (95% CI 41.42-59.38) in low T cell group. There was no significant difference of neutrophil and platelet recovery time between two groups ($P>0.05$). The lymphocyte recovery time in high T cell group was longer than that in low T cell group ($P=0.0002$) (Fig. 1).

The cumulative incidence of sustained neutrophil recovery (day 12) was 100% in low T groups and was 83.3% in high T groups. The cumulative incidence of sustained platelet recovery (day 15) was 100% in low T groups and was 90% in high T groups. There was no significant difference of sustained neutrophil and platelet recovery between two groups ($P>0.05$). Patients receiving high T cell in grafts had a higher cumulative incidence of sustained lymphocyte recovery (3 months) compared with low T cells in grafts (100 vs. 44%) ($P<0.05$) (Fig. 2).

CMV reactivation. All patients had CMV reactivation at a median of 28 days (range, 14-42) (Fig. 3). In multivariate analysis, donor sex and age as well as the CMV seropositive status of the recipient and donor was not associated with the CMV reactivation.

GVHD. The cumulative incidence of grade II or above acute GVHD was higher in high T groups than that in low T groups ($P<0.05$). There was no significant difference of the cumulative incidence of chronic GVHD between two groups ($P>0.05$) (Fig. 4).

Table I. Baseline characteristics of the two study groups.

Transplant variables	Low CD3+ T cells (n=15)	High CD3+ T cells (n=15)	P-value
Recipient			
Age (years), median (range)	33 (10-52)	32 (14-55)	>0.05
CMV serology, neg/pos	1/14	2/13	>0.05
Disease, acute leuk/other	14/1	12/3	>0.05
Disease status, early/other	10/5	11/4	>0.05
Donor			
Age (years), median (range)	28 (17-50)	32 (22-47)	>0.05
CMV serology, neg/pos	0/15	1/15	>0.05
Recipient/donor			
Sex (R:D), M:F/other	4/11	2/13	>0.05
ABO mismatch, minor/major	1/0	1/1	>0.05
Transplant			
Conditioning, MAC/RIC	14/1	12/3	>0.05
ATG, no/yes	0/15	0/15	>0.05
PBSC graft			
TNC ($x10^8$/kg), median (range)	358.53 (150.67-454.93)	430.5 (260.9-619.87)	>0.05
CD34+ ($x10^6$/kg), median (range)	9.17 (5.01-17.43)	7.16 (2.14-10.91)	>0.05
CD3+ ($x10^6$/kg), median (range)	244.7 (170.2-299.7)	366.6 (326.2-623.4)	>0.05

CMV, cytomegalovirus; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; HSCT, hematopoietic stem cell transplantation; CR, complete remission; MRD, minimal residual disease; MAC, myeloablative conditioning; RIC, reduced intensity conditioning; ATG, rabbit anti-human thymocyte immunoglobulin; TNC, total nucleated cells.

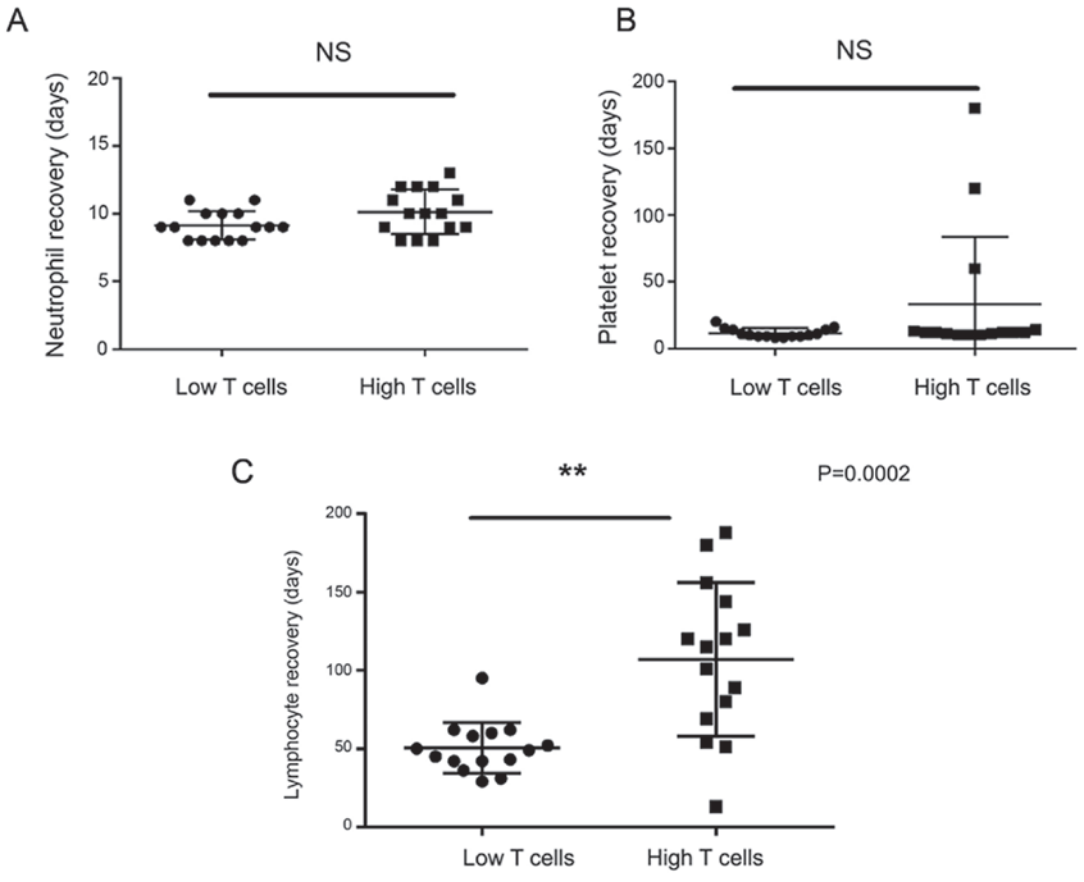

Figure 1. Neutrophil, platelet and lymphocyte engraftment of two groups. (A) Neutrophil recovery; (B) platelet revcovery and (C) lymphocyte recovery. NS, not significant. **P>0.05.

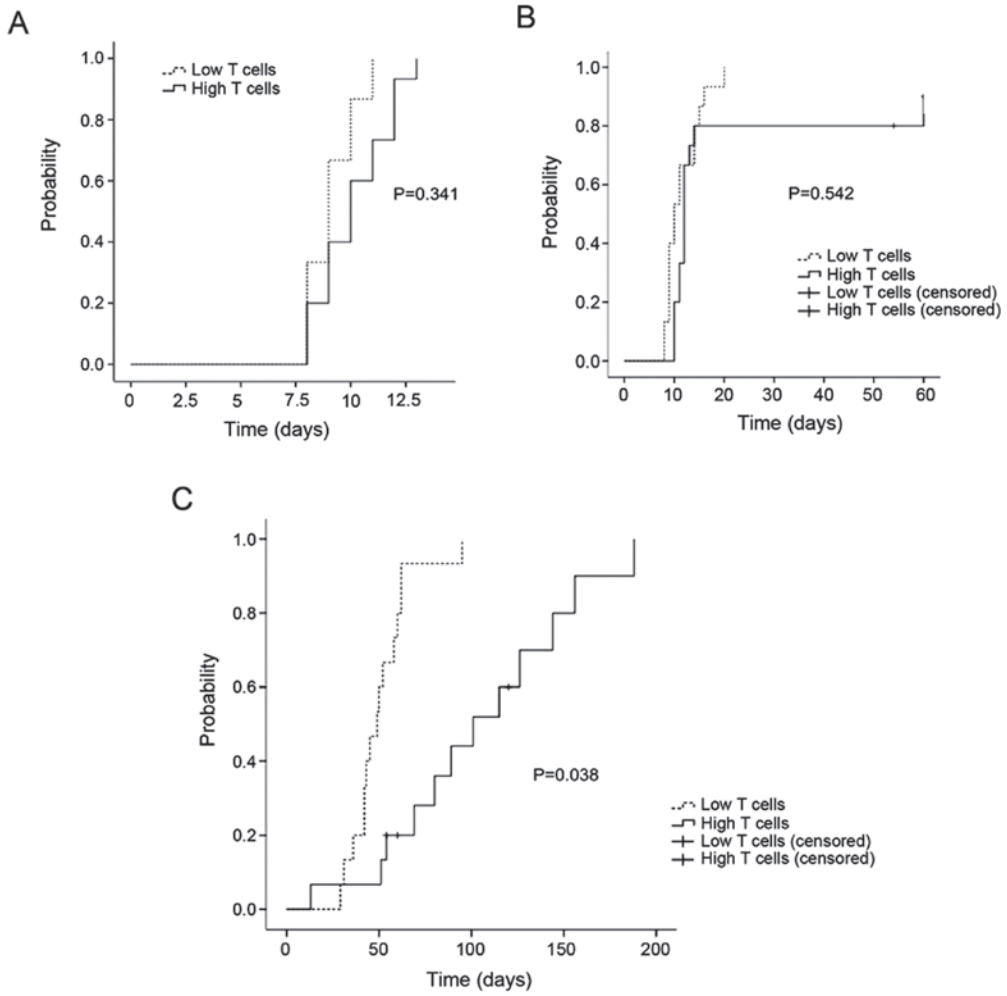

Figure 2. The cumulative incidence of sustained hematopoietic recovery. (A) neutrophil recovery; (B) platelet recovery and (C) lymphocyte recovery.

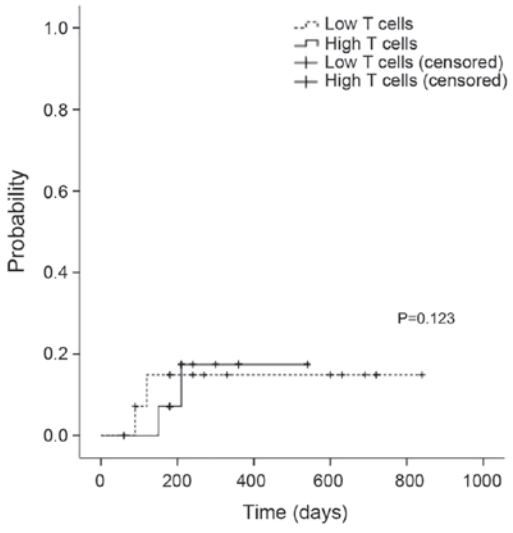

Figure 3. CMV reactivation between two groups. CMV, cytomegalovirus.

Disease relapse and TRM. Six patients relapsed, the cumulative incidence of relapse at 1 year was 15% in low T cell group and 17.5% in high T cell group, there was no significant difference of disease relapse rate between two groups (P>0.05) (Fig. 5). Nine patients died, including five died of primary disease relapse, two died of pulmonary infection, and two died of refractory acute GVHD.

Progress free and overall survival. The 1-year PFS was 71.1% in low T cell and 63.2% in high T cell group. The 2 years PFS was 71.1% in low T cell and 42.1% in high T cell group. There was significant difference of the one and two years of PFS between two groups (P=0.025). The estimated OS at 1 year was 86.7% in low T cell and 92.9% in high T cell group. The estimated OS at 3 years was 69.5% in low T cell and 48.8% in high T cell group. There was significant difference of OS between two groups (P=0.042) (Fig. 6).

Discussion

HSCT remains the curative treatment for patients with non-malignant and malignant hematological disorders. HSCT from HLA-mismatched haploidentical donors was an option for patients lacking HLA-matched donors. A major obstacle to haploidentical HSCT was high rates of graft rejection and GVHD. Alloreactive lymphocytes of the graft can mediate a potentially life-threatening GVHD due to HLA dissimilarity (5-8). In order to decrease the lethally acute GVHD induced by alloreactive lymphocyte in the graft, various approaches to prevent GVHD are being investigated, including

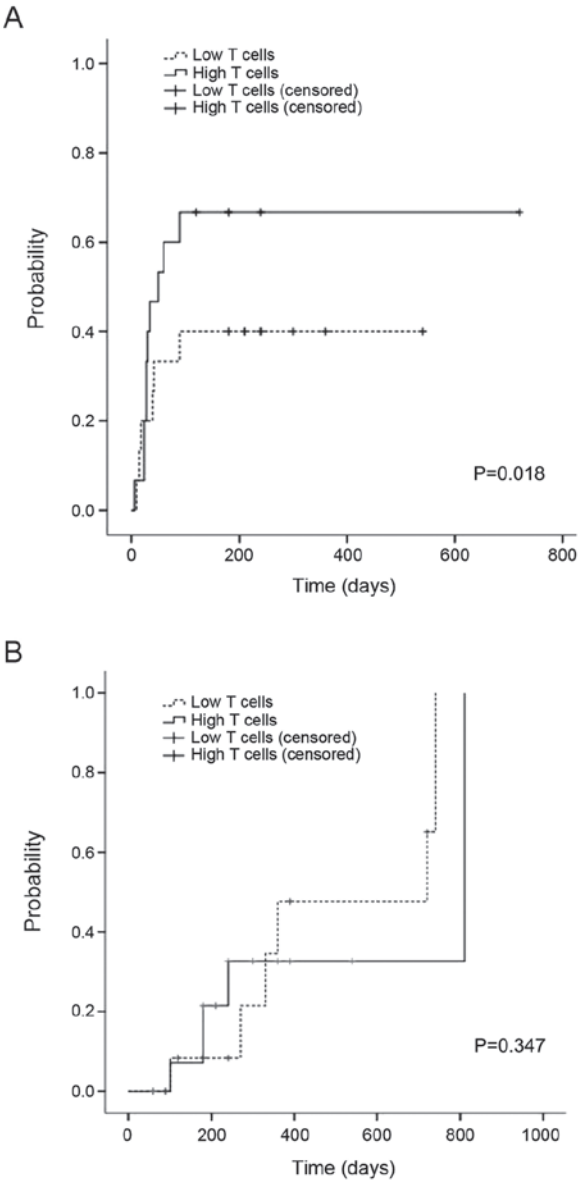

Figure 4. Acute and chronic GVHD in two groups. (A) Incidence of acute GVHD. (B) Chronic incidence of chronic. GVHD, graft versus host disease.

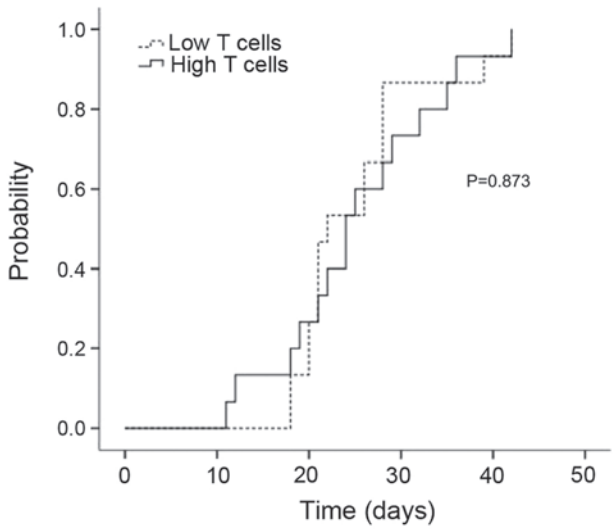

Figure 5. Relapse rate in two groups.

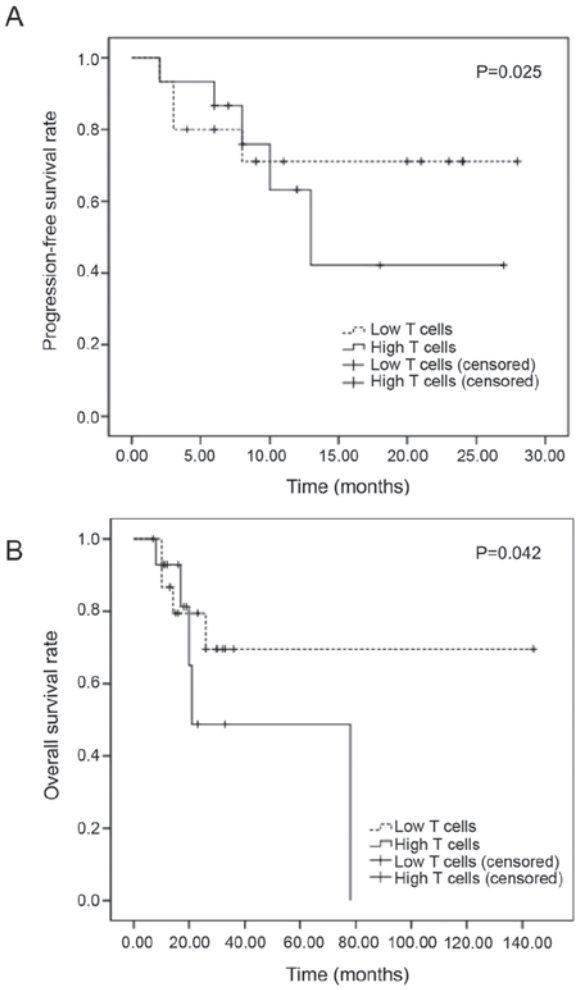

Figure 6. Progress free survival and overall survival in two groups. (A) Progress free survival. (B) Overall survival.

in vitro T cell depletion of bone marrow or peripheral blood stem cells (PBSCs) or, more recently, *in vivo* T cell depletion approaches using either granulocyte colony-stimulating factor (G-CSF)-mobilized bone marrow in combination with PBSCs and anti-thymocyte globulin or the administration of high-dose cyclophosphamide after transplantation of haploidentical bone marrow-derived progenitor cells. These trials *in vitro* or *in vivo* depletion of donor T cells showed initially promising results by marked reduction of risk of GVHD even without the use of post-transplant pharmacological GVHD prophylaxis (9-13). Therefore, however, this was associated with an increased risk of disease relapse, impaired immune recovery and also an increased incidence of graft failure was observed, in both matched and unmatched donors, which suggesting that donor T cells in graft act as a double-edged sword, it may help eradicate malignant clones and counter balance the ability of residual recipient T cells (surviving conditioning regimen) to reject the graft (14).

How to balance the function of donor T cells in the graft and how many T cells in the graft is better for recipients, it is still uncertain. One study found that more than $0.2x10^6$/kg $CD3^+$ T cells in the graft was an important factor for sustained engraftment in CD34 positive selected G-PBSC transplantation (1). Several studies found that higher CD3+ T cell dose

is independently associated with more severe acute GVHD in HLA matched HSCT (15-18), but Kałwak *et al* (3) reported that pediatric patients received ≥4x10^8 $CD3^+$ T cells/kg had a better overall survival, without an increased risk of severe GVHD in HLA matched HSCT. In haploidentical stem cell transplantation, Pastore *et al* (19) found that patients with doses of CD3+ T cells above the median 177x106/kg in bone marrow combined with peripheral blood stem cell grafts had a significantly better overall survival without increase the acute GVHD. But rare study was reported about the effects of $CD3^+$ T cells in the peripheral blood stem cell grafts only on the hematopoietic reconstitution, GVHD and overall survival. In this study, we found that high $CD3^+$ T cells in peripheral blood stem cell graft will increase the rate of acute GVHD, delay the lymphocyte recovery and decrease the overall survival in haploidentical stem cell transplantation.

Which subset of T cells was the keycells that induces a GVHD. It is reported that low CD3+/Treg ratio in graft will decrease the incidence of acute GVHD in HLA matched HSCT, because Treg cells was able to mediate protective effects against acute GVHD and to maintain an optimal microenviroment for the reconstitution of functional immunity and will improved the hematopoietic reconstitution and improve the overall survival of the patients (20). In this regard, Rezvani *et al* (21) determined that increased frequencies of $CD4^+Foxp3^+$ Treg cells in the peripheral blood of the donor negatively correlated with the incidence of GVHD in the graft recipient. Several subsequent studies confirmed this correlation in recipients of HLA-identical sibling and unrelated donor stem cell grafts indicating that hematopoietic stem cell graft content appears to modulate GVHD severity (21). In a prospective study found that in haploidentical HSCT patients with infusion of >0.22x10^8 $CD4^+CD45RA^+CD62L^+$ cells infused/kg had an increased risk of grade II-IV acute GVHD and the risk of chronic GVHD was increased in the patients receiving >0.45x$10^8$$CD4^+CD45RA^+$ cells infused/kg (22). It suggested that allo-depletion of naïve $CD4^+$ T cells contributes to alleviating GVHD, especially in patients receiving haploidentical allografts. Preclinical models showed that both CD4+ and CD8+ T cells are capable of mediating lethal GVHD in HLA-incompatible transplants. Mohty *et al* (22) reported that the CD8+ T cell dose infused was the only parameter associated with the risk of a GVHD (P=031; RR=1.96) after a multivariate analysis. But in haploidentical HSCT, Chang *et al* (23) reported that researchers from China found that patients with a higher CD4/CD8 ratio in the G-CSF mobilized marrow harvests (≥1.16) had a survival disadvantage, a significantly increased risk of aGVHD grades II-IV and a trend towards relapse (23). In this study, patients infused with higher $CD3^+$ T cells had higher incidence of acute GVHD, it may due to high $CD3^+$ T cells contains more $CD4^+$ and $CD8^+$ T cells which would induce acute GVHD. When patients had acute GVHD, the immunosuppression drugs will be used and lymphocyte recovery delayed which resulted in high incidence of infection and non-relapse mortality.

This study has many limitations that cell subsets in the graft cell is not detected and different subsets such as NK, B and DC cells may also have different impacts on hematopoietic engraftment, immune recovery and long-term outcome.

Overall, there are still controversial about different effects of the $CD3^+$ T cells in the grafts on transplant outcomes, because patients with different conditioning regimens, allografts origin, GVHD prophylaxis, and different underlying disease may have different complications and outcomes of HSCT. These variables should be considered to carefully assess the $CD3^+$ T graft content and tailor the cell dose infused in order to reduce complications and improve the overall survival and disease progress survival.

Acknowledgements

Not applicable.

Funding

The present study was supported by the National Nature Science Fund of China (grant no. 81600179) and the Natural Science Basic Research of Shaanxi Province (grant no. 2019JM-564).

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

YZ and XW designed the study and wrote the initial draft of the manuscript. XW and PH contributed to refining the figures. CG, CS, YC, JX, HZ and MZ contributed to the analysis and interpretation of data. YC, JX and HZ verified the analytical methods. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Ethics Comittee of the First Affiliated Hospital of Xi'an Jiaotong University (Xi'an, China). All of patients and donors, or their legal guardians, provided written informed consent in accordance with the Declaration of Helsiniki.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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