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Efficacy of Haploidentical Allogeneic Hematopoietic Stem Cell Transplantation for 40 Cases with Severe Aplastic Anemia

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Abstract

Objective: To investigate the efficacy and prognosis of Haploidentical Allogeneic Hematopoietic Stem Cell Transplantation (Haplo-HSCT) for Severe Aplastic Anemia (SAA).

Methods: The clinical data of 40 SAA cases (29 with SAA-I, 9 with SAA-II, 2 with posthepatisis SAA) with Haplo-HSCT from September 2013 to February 2018 were retrospectively analyzed. 33 SAA patients received peripheral blood hematopoietic stem cells, 7 SAA patients combined with bone marrow hematopoietic stem cells. The conditioning regimen contained cyclophosphamide, fludarabine and antithymocyte globulin, with or without busulfan or low dose total body irradiation. Cyclosporin A, short-term methotrexate and mycophenolate mofetil were used for preventing Graft Versus Host Disease (GVHD).The median counts of Mononuclear Cell (MNC) and CD34+ stem cell were 5.3 (range: 2.0 to 13.5) × 10^8/kg and 5.6 (range: 1.6 to 15.9) × 10^6/kg, respectively.

Results: Among 40 SAA cases, hematopoiesis reconstitution was achieved in 36 cases (90.0%). The median times for myeloid engraftment and platelet engraftment were 15 (range: 10 to 25) and 17 (range: 10 to 58) days, respectively. The incidence of Acute Graft-Versus-Host Disease (aGVHD) was 35.0% ± 6.8%, grades II-IV aGVHD 20.0% ± 3.4%, and grades III-IV aGVHD 10.0% ± 6.1%. The incidence of Chronic GVHD (cGVHD) was 23.0% ± 7.4%, extensive cGVHD 11% ± 3.7%. 28 (70.0%) SAA cases alived at median follow-up time of 353 (30-1226) days, the cumulative Overall Survival (OS) was 67.8% ± 7.8%, and the average time was 883 ± 82 days, Transplantation-Related Mortality (TRM) within 100 days was 10.0% ± 3.1%.

Conclusion: Haplo-HSCT was an effective option for SAA patients; however, it needs to be studied in a large number of cases for enhancing overall survival.

Keywords: Severe aplastic anemia; Haploidentical allogeneic hematopoietic stem cell transplantation; Human leukocyte antigen

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Introduction

Severe Aplastic Anemia (SAA) is a life-threating disease, characterized by acute onset, rapid progression, high risk and poor prognosis. The natural history is only 3 to 6 months [1]. Allogeneic Haematopoietic Stem Cell Transplantation (HSCT) is the available curative approach. HLA Matched Sibling Donor HSCT (MSD HSCT) is the preferred treatment, with fast hematopoietic reconstitution, low incidence of clonal diseases and high long-term survival rate. However, only 25% to 30% of patients have a matched sibling [2]. For the patients without a MSD, Immunosuppressive Therapy (IST) is the first-line treatment, which is less effective and higher replase rate. In addition, clonal evolution can occur [3]. Matched Unrelated Donor HSCT (MUD HSCT) provides another possibility for the treatment of SAA, but it is difficult to find a matched donor from the Chinese bone marrow bank, and takes a long time. Most of the time it delays the optimum opportunity of transplantation [4]. Haploidentical HSCT (Haplo-HSCT) has solved the problem of finding suitable donors. Several studies have indicated that among hematological malignances in several centers, Haplo-HSCT and MSD HSCT is equivalent, and the overall survival is no significant difference [5-6]. Recent studies have gradually been carried out on the Haplo-HSCT with SAA, the donor is readily available, parents and compatriots are with good compliance [7-10]. To investigate the efficacy and prognosis of Haplo-HSCT for SAA, we analyze 40 SAA cases with Haplo-HSCT from

September 2013 to February 2018 in our centre.

Methods

Patients

About 40 SAA cases with Haplo-HSCT at the First Affiliated Hospital of Zhengzhou University from September 2013 to February 2018 are enrolled (Table 1).

Conditioning regimen

Conditioning regimen of 29 cases is FC+ATG: fludarabine 30 mg/(m2•d) × 4 d, cyclophosphamide 50 mg/(kg•d) × 4 d, Rabbit Antithymocyte Globulin (r-ATG) 2.5 mg/(m2•d) × 4 d. About 7 cases were added Busulfan (BU) (0.8 mg/kg q6h) × 3 d. About 4 cases were added Total Body Irradiation (TBI) 2 Gy to 5 Gy.

Hematopoietic stem cell mobilization collection

The donor received Recombinant Human Granulocyte Colony-Stimulating Factor (rhG-CSF) at 5 to 10 μg/kg•d × (4-6) d. On the 5th day, our center collected Peripheral Blood Hematopoietic Stem Cells (PBSC) and bone marrow hematopoietic stem cells from the posterior superior iliac spine under local anesthesia (300 ml to 400 ml routinely collected in our centre). The median counts of MNC and CD34+ stem cell were 5.3 (range: 2.0 to 13.5) × 10^8/kg and 5.6 (range: 1.6 to 15.9) × 10^6/kg, respectively.

Prophylaxis and treatment of GVHD

Cyclosporin A, short-term methotrexate and mycophenolate mofetil were used for preventing Acute Graft-Versus-Host-Disease (aGVHD): CsA (3 mg/(kg•d), intravenous injection) was administered from day -9 to day +28, switched to oral after recovery of gastrointestinal function, maintaining blood concentration of 200 μg/L~400 μg/L: MTX (15 mg/m^2) was administered intravenously on day +1, followed by a dose of 10 mg/m^2 on days +3,+6; MMF (15 mg/kg q12h) was administered orally from day -9 to day +75. In some patients, basiliximab and infusion of mesenchymal stem cells were used to reduce the incidence of GVHD.

Supportive treatment

All patients entered the sterile laminar airflow room. Alprostadil was used to prevent hepatic Veno Occlusive Disease (VOD). Regular peripheral blood tests were performed, and the irradiated blood was infused actively according to the results, maintaining hemoglobin ≥ 80 g/L, platelets ≥ 20 × 10^9/L. Blood cultures were carried out when patients got fever and empirical antibiotics were used. Antibiotics were adjusted according to pathogenic and imaging results. Considering the fungal infections, antifungal drugs such as itraconazole and voriconazole were taken.

Hematopoietic reconstruction and engraftment

Peripheral blood neutrophil absolute value (ANC) for 3 consecutive days' ≥ 0.5 × 10^9/L was myeloid engraftment. Platelet (PLT) infusion free and for 3d ≥ 20 × 10^9/L was platelet engraftment. The chimerism of the donor and recipient was monitored by blood type test, DNA Short Tandem Repeat (STR) test and Fluorescence *in Situ* Hybridization.

Assessments of transplantation

Indicators include Overall Survival (OS), Event-Free Survival (EFS), the time of myeloid engraftment and platelet engraftment, Transplantation-Related Mortality (TRM), Primary Graft Failure (PGF), Graft Failure (GF), aGVHD, Chronic Graft-Versus-Host-Disease (cGVHD).

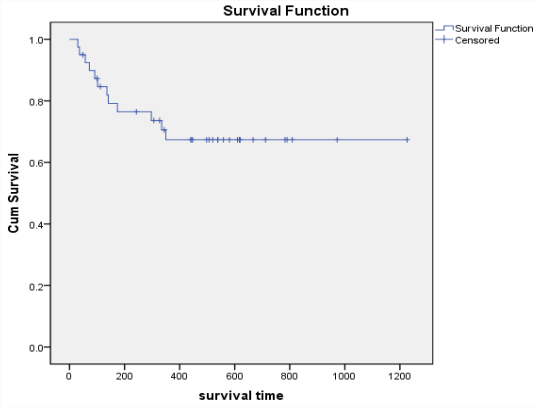

Figure 1: Survival curve, cumulative overall survival rate is 67.8% ± 7.8%.

Statistical analysis

All patients were followed by further consultation or telephone. The dead line of follow-up was February 28, 2018 or the time of death. The median time of follow-up was 353 (30-1226) days. Data analysis was performed using statistical software SPSS 21.0. The overall survival rate and event-free survival rate were calculated by Kaplan-Meiter method. Log-rank test was used to compare the rate. Multivariate logistic analysis used the COX regression analysis. If $P<0.05$, it suggested that the difference was statistically significant.

Results

Hematopoietic reconstitution

About 36 patients (90.0%) were achieved myeloid engraftment and hematopoietic reconstitution. The median time of myeloid engraftment and platelet engraftment were 15 (10 to 25) days and 17 (10 to 58) days. The ANC of one case was 0 at the day +20, and no focal hyperplasia was found in the bone marrow, considering PGF, which was performed the secondary transplantation. But this case died of severe sepsis at the day +30. One case's chimerism rate was 41.38% after transplantation in one month and blood type remained unchanged at the day +38, which is considered the delayed graft failure, he did not accept the secondary transplantation, died due to the severe lung infection on the day +72. There was one case whose platelet was still low on the day +29, considered as graft failure. The secondary transplantation was performed but died of severe sepsis on the day +335. There was one case showing myeloid engraftment and platelet engraftment on the day +15. The platelet decreased from the day +18. No secondary transplantation was performed. But he purchased the Eltrombopag which maintain the platelet to normal.

The incidence of GVHD

The incidence of aGVHD, cGVHD was 35.0% ± 6.8%, 23.0% ± 7.4% and the incidence of extensive cGVHD was 11% ± 3.7%. The incidence of II-IV, III-IV was 20.0% ± 3.4% and 10.0% ± 6.1%. One case died of digestive tract GVHD and lower digestive tract hemorrhage on the day +174.

Post-transplant infection

When occurring sepsis, digestive tract and pulmonary infections, and so on, the anti-infective treatment is inefficient after 3 days or during treatment infection-related mortality appeared which are classified as severe infection. About 10 cases (25.0%) caused severe infection after transplantation. About 7 cases (17.5%) were sepsis. About 10 cases (25.0%) were pulmonary infection. About 18 cases

Table 1: Patient characteristics.

Variable	Values (N=40)
Gender (cases, male/female)	29/11
Age [year, M(range)]	13 (4-46)
Time (from diagnosis to transplantation) [day, M (range)]	60 (15-3650)
Type of diseases [cases (%)]	
SAA-I	29 (72.5)
SAA-II	9 (22.5)
SAA after hepatitis	2 (5)
HLA type [cases (%)]	
5/10	28 (70.0)
>5/10	12 (30.0)
Donors [cases (%)]	
Sibling	9 (22.5)
father	22 (55.0)
mother	7 (17.5)
child	2 (5)
ABO [cases (%)]	
Matched	19 (47.5)
Not Matched	21 (52.5)
Graft type [cases (%)]	
Peripheral blood	33 (82.5)
Peripheral blood+bone marrow	7 (17.5)

(45.0%) were invasive fungal disease. About 2 cases (5.0%) were urinary tract infection. There were 4 cases (10.0%) of perianal infection and 5 cases (12.5%) of digestive tract infection, including 3 cases (7.5%) of spontaneous peritonitis.

Other complications

The incidence of EBV, CMV, and BKV were 22.5% (9/40), 40.0% (16/40), and 7.5% (3/40), respectively. They were treated by antiviral drugs as ganciclovir and foscarnet sodium and recovered. About 9 cases got hemorrhagic cystitis after transplantation, and were controlled after hydration, alkalization and symptomatic treatment. There was 1 case of tuberculosis, 3 cases of herpes zoster, 1 case of anaphylactoid purpura. They were all recovered after treatment. One patient developed the hepatic Veno Occlusive Disease (VOD) on the day +3. After treated by alteplase and low molecular heparin calcium, the symptoms were relieved.

Efficacy and follow-up

To the end of follow-up, 28 patients (70.0%) survived among 40 patients. The median follow-up time was 353 (30 to 1226) days and the overall survival rate was 67.8% ± 7.8%. The survival curve is shown in Figure 1. About 10 cases died of severe infection, 1 case died of digestive tract GVHD and lower digestive tract hemorrhage. One case died of upper digestive tract hemorrhage. The average survival time was 883 ± 82 days. The event-free survival rate was 55.2% ± 8.2%. Five cases died within 100 days. The transplant-related mortality within 100 days was 10.0% ± 3.1%. Four of them died of severe infection and one died of upper digestive tract hemorrhage.

OS' affecting factors

OS is significantly correlation with the disease type, the count of D34+ cell, GVHD, severe infection, virus infection and fungal infection (P were 0.000, 0.045, 0.037, 0.000, 0.008, 0.005, respectively). Univariate analysis is shown in Table 2.

Table 2: Univariate analysis.

Variable	Cases	Overall survival rate (100%)	X^2	P
GVHD			4.351	0.037
yes	15	47.7 ± 12.9		
no	25	82.9 ± 7.8		
Severe infection			22.777	0
yes	10	20.0 ± 12.6		
no	30	84.4 ± 7.2		
Virus infection			7.097	0.008
yes	23	51.8 ± 10.5		
no	17	91.7 ± 8.0		
Invasive fungal disease			8.001	0.005
yes	18	43.8 ± 13.0		
no	22	85.2 ± 7.9		
Disease type			13.091	0
SAA- I	31	79.0 ± 7.7		
SAA- II	9	27.8 ± 16.2		
Count of CD34+ cell			4.037	0.045
≤ 2 × 10^6/kg	5	40.0 ± 21.9		
>2 × 10^6/kg	35	72.2 ± 8.0		

Table 3: Multivariate logistic analysis: Disease type and severe infection are independent risk factors affecting the overall survival of haploidentical hematopoietic stem cell transplantation in SAA.

	P	OR	95% CI
Disease type	0.045	1.184	0.035-0.964
Count of CD34+ cell	0.848	1.19	0.201-7.029
GVHD	0.267	0.468	1.123-1.788
Severe infection	0.044	0.218	0.050-0.959
Viral infection	0.309	0.288	0.026-3.168
Invasive fungal disease	0.052	0.213	0.045-1.016

Discussion

SAA is an acquired marrow failure syndrome characterized of peripheral pancytopenia and bone marrow hematopoietic dysfunction, which caused by multiple etiology and multiple pathogenesis [11]. For patients without MSD/MUD and failure to respond to Immunosuppressive Therapy (IST), we can choose Umbilical Cord Blood Transplantation (UCBT) or Haplo-HSCT. However, the UCDT costs more and the rate of graft failure is higher [12]. In recent years, Haplo-HSCT has become a new approach to the treatment of SAA [13-15]. Almost every patient can find a suitable donor, which is time-saving and less expensive. But Haplo-HSCT also encounters some challenges. GF or delayed GF is the major challenge. In recent years, the improvement of conditioning regimen, the selection of grafts, the increase of CD34+ cells and the application of high-dose post-transplantation cyclophosphamide, has solved the problems of graftment difficulties and reduction of post-transplant related complications [16,17]. Classical conditioning regimen for SAA is cyclophosphamide and antithymocyte globulin [18]. Basised on it, Fludarabine (FLU), Busulfan (BU) and total body irradiation are added, which intensify the immunosuppressive effect. It is

expected to completely remove residual hematopoietic stem cells, and to reduce the probability of rejection, and to facilitate the graftment of hematopoietic stem cells which could improve overall survival [19-21]. FLU is a purine analog with cytotoxicity and immunosuppressive effect, it can intensify conditioning regimen while promote graftment [22]. BU is an alkylating agent that enhances the immunosuppressive function of cyclophosphamide to promote stable graftment [23]. Based on FCA, in our study 7 cases used BU and 4 cases used TBI. Except that one case with PFG added busulfan undergo the secondary implantation. The others achieved complete graftment. Granulocyte Colony-Stimulating Factor (G-CSF) mobilizes peripheral blood and bone marrow to increase the number of stem cells and increase the count of bone marrow mesenchymal stem cells, which can accelerate hematopoietic reconstitution and increase the graftment rate [24]. In our study, 7 patients with peripheral blood combined with bone marrow hematopoietic stem cell transplantation achieved primary graftment. However, the sample size of this study is small, and there are many confounding factors, large sample research still need to be conducted. GVHD is another major challenge. FLU can reduce GVHD caused by small doses of residual donor cells while increasing graftment [25]. The use of G-CSF to mobilize peripheral blood and bone marrow causes T cells to transform from Th1 to Th2, which can prevent the occurance of aGVHD [26]. Our study uses a conditioning regimen containing FLU and G-CSF to mobilize hematopoietic stem cells, thereby reducing the incidence of GVHD. Basiliximab is an immunosuppressant of T cell activation and proliferation induced by IL-2. Mesenchymal stem cell contains various stromal cells. It can prevent GVHD by inhibiting immunity with T cell or releasing soluble cytokines [27]. In our center, based on Cyclosporine A (CSA) and short-term Methotrexate (MTX) and Mycophenolate Mofetil (MMF) as a triple combination, we use basiliximab and mesenchymal stem cells to prevent GVHD. When acute or severe GVHD occurs, it is preferred to choose methylprednisolone 2 mg/(kg•d). If it does not work, cyclosporine A is instead by tacrolimus or basiliximab, mesenchymal stem cells is also widely used. In this study, the incidence of aGVHD is 35.0% ± 6.8%, the incidence of II-IV and III-IV is 20.0% ± 3.4% and 10.0% ± 6.1%. The incidence of cGVHD is 23.0% ± 7.4%, the incidence of extensive cGVHD is 11% ± 3.7%. One case died of digestive tract GVHD and lower digestive tract hemorrhage on the day +174. The incidence of GVHD in this study is lower than the previous reports. cGVHD is significantly lower than the related reports, which may be related to the effective control of aGVHD after treatment. This indicates that CSA with short-term MTX and MMF and basiliximab and mesenchymal stem cells can effectively reduce the incidence of GVHD. The prevention and treatment of aGVHD is beneficial to reduce cGVHD. Due to bone marrow failure in SAA patients, their granulocytes are in a state of deficiencies for a long time, and immunologic function is poor. So they are highly susceptible to inflammation and it is difficult to control. The patients with Haplo-HSCT are often treated by a large number of potent immunosuppressive agents in a short period and still need to take oral immunosuppressive agents for a long term after transplantation. Therefore, infection is one of the fatal causes in SAA patients. In this study, the incidence of bacterial infection and invasive fungal disease is 72.5% (29/40). Of the 12 died cases, 10 (25.0%) of them are combination of blood infection and pulmonary infection when waiting for MUD, so the optimal opportunity of transplantation is delayed. Univariate analysis shows that the OS rate of patients with severe infection (20.0 ± 12.6) is significantly lower than that of patients without severe infection (84.4 ± 7.2) (P=0.000). Multivariate logistic analysis shows that severe infection are independent risk factors affecting the overall survival of Haplo-HSCT in SAA (p=0.044). Therefore, the choice of transplantation opportunity, strict sterile operation, good environmental protection, and active infection control are extremely important for prevention and treatment of infection during transplantation. Virus infection is also a major complication after transplantation. The incidences of EBV, CMV, and BKV infection in this study are 22.5% (9/40), 40.0% (16/40), and 7.5% (3/40), respectively. After antiviral treatment with ganciclovir and foscarnet sodium, the virus replication recovers to normal. Two patients developed Posttransplant Lymphoproliferative Disorders (PTLD) due to Epstein-Barr virus infection. One of them developed diffuse large B cell lymphoma on the day +61, and received a chemotherapy regimen of CHOP. Then the patient returned to the local hospital but didn't receive the chemotherapy. Univariate analysis shows there is a correlation between virus infection and overall survival rate (P=0.008). Therefore, intensive monitoring is necessary. Once the viremia is detected, the preemptive treatment is given immediately, which may help reduce the incidence of lethal viral infection.

Conclusion

Haplo-HSCT is a safe and effective alternative treatment for SAA patients who have no MSD, MUD and who are not treated with immunosuppressive agents. But due to the small number of cases in this study group and that some patients have shorter follow-up time; further large-scale prospective studies are still needed in order to deeply confirm the efficacy of haploid hematopoietic stem cell transplantation in the treatment of severe aplastic anemia.

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References

1. Young NS, Kaufman DW. The epidemiology of acquired aplastic anemia. Haematologica. 2008;93(4):489-92.
2. Miano M, Dufour C. The diagnosis and treatment of aplastic anemia: a review. Int J Hematol. 2015;101(6):527-35.
3. Bacigalupo A, Giammarco S, Sica S. Bone marrow transplantation versus immunosuppressive therapy in patients with acquired severe aplastic anemia. Int J Hematol. 2016;104(2):168-74.
4. Devillier R, Dalle JH, Kulasekararaj A, D'aveni M, Clément L, Chybicka A, et al. Unrelated alternative donor transplantation for severe acquired aplastic anemia: a study from the French Society of Bone Marrow Transplantation and Cell Therapies and the EBMT Severe Aplastic Anemia Working Party. Haematologica. 2016;101(7):884-90.
5. Ciurea SO, Zhang MJ, Bacigalupo AA, Bashey A, Appelbaum FR, Aljitawi OS, et al. Haploidentical transplant with posttransplant cyclophosphamide vs matched unrelated donor transplant for acute myeloid leukemia. Blood. 2015;126(8):1033-40.
6. Santoro N, Ruggeri A, Labopin M, Bacigalupo A, Ciceri F, Gülbaş Z, et al. Unmanipulated haploidentical stem cell transplantation in adults with acute lymphoblastic leukemia: a study on behalf of the Acute Leukemia Working Party of the EBMT. J Hematol Oncol. 2017;10(1):113.
7. Im HJ, Koh KN, Choi ES, Jang S, Kwon SW, Park CJ, et al. Excellent outcome of haploidentical hematopoietic stem cell transplantation in children and adolescents with acquired severe aplastic anemia. Biol Blood Marrow Transplant. 2013;19(5):754-9.

8. Clay J, Kulasekararaj AG, Potter V, Grimaldi F, McLornan D, Raj K, et al. Nonmyeloablative peripheral blood haploidentical stem cell transplantation for refractory severe aplastic anemia. Biol Blood Marrow Transplant. 2014;20(11):1711-6.
9. Wang Z, Zheng X, Yan H, Li D, Wang H. Good outcome of haploidentical hematopoietic SCT as a salvage therapy in children and adolescents with acquired severe aplastic anemia. Bone Marrow Transplant. 2014;49(12):1481-5.
10. Xu LP, Jin S, Wang SQ, Xia LH, Bai H, Gao SJ, et al. Upfront haploidentical transplant for acquired severe aplastic anemia: registry-based comparison with matched related transplant. J Hematol Oncol. 2017;10(1):25.
11. Bacigalupo A. How I treat acquired aplastic anemia. Blood. 2017;129(11):1428-36.
12. Kuwatsuka Y, Kanda J, Yamazaki H, Mori T, Miyamura K, Kako S, et al. A Comparison of Outcomes for Cord Blood Transplantation and Unrelated Bone Marrow Transplantation in Adult Aplastic Anemia. Biol Blood Marrow Transplant, 2016;22(10):1836-43.
13. Gao L, Li Y, Zhang Y, Chen X, Gao L, Zhang C, et al. Long-term outcome of HLA-haploidentical hematopoietic SCT without *in vitro* T-cell depletion for adult severe aplastic anemia after modified conditioning and supportive therapy. Bone Marrow Transplant. 2014;49(4):519-24.
14. Esteves I, Bonfim C, Pasquini R, Funke V, Pereira NF, Rocha V, et al. Haploidentical BMT and post-transplant Cy for severe aplastic anemia: a multicenter retrospective study. Bone Marrow Transplant. 2015;50(5):685-9.
15. Xu LP, Zhang XH, Wang FR, Mo XD, Han TT, Han W, et al., Haploidentical transplantation for pediatric patients with acquired severe aplastic anemia. Bone Marrow Transplant. 2017;52(3):381-7.
16. DeZern AE, Zahurak M, Symons H, Cooke K, Jones RJ, Brodsky RA. Alternative Donor Transplantation with High-Dose Post-Transplantation Cyclophosphamide for Refractory Severe Aplastic Anemia. Biol Blood Marrow Transplant. 2017;23(3):498-504.
17. Wang L, Wu YM, Cao YB, Li XH, Xu LX, Wang HT, et al. [Hematopoietic Reconstitution and Prognosis of HLA Matched and Haploidentical Hematopoietic Stem Cell Transplantation Using Modified FC/ATG Conditioning for Treatment of Severe Aplastic Anemia]. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2016;24(6):1817-23.
18. Champlin RE, Perez WS, Passweg JR, Klein JP, Camitta BM, Gluckman E, et al. Bone marrow transplantation for severe aplastic anemia: a randomized controlled study of conditioning regimens. Blood. 2007;109(10):4582-5.
19. Sun C, Lin X, Huang Y, Song C, Tao Y, Tu S, et al. [Fludarabine-based increased-intensity conditioning regimen for allogeneic hematopoietic stem cell transplantation in acquired severe aplastic anemia]. Zhonghua Xue Ye Xue Za Zhi. 2014;35(3):221-4.
20. Bacigalupo A, Socie' G, Lanino E, Prete A, Locatelli F, Locasciulli A, et al. Fludarabine, cyclophosphamide, antithymocyte globulin, with or without low dose total body irradiation, for alternative donor transplants, in acquired severe aplastic anemia: a retrospective study from the EBMT-SAA Working Party. Haematologica. 2010;95(6):976-82.
21. Ommati LV, Rodrigues CA, Silva AR, Silva LP, Chaufaille ML, Oliveira JS. A retrospective comparison of cyclophosphamide plus antithymocyte globulin with cyclophosphamide plus busulfan as the conditioning regimen for severe aplastic anemia. Braz J Med Biol Res. 2009;42(3):244-50.
22. Kang HJ, Hong KT, Lee JW, Kim H, Park KD, Shin HY, et al. Improved Outcome of a Reduced Toxicity-Fludarabine, Cyclophosphamide, plus Antithymocyte Globulin Conditioning Regimen for Unrelated Donor Transplantation in Severe Aplastic Anemia: Comparison of 2 Multicenter Prospective Studies. Biol Blood Marrow Transplant. 2016;22(8):1455-9.
23. Dulley FL, Vigorito AC, Aranha FJ, Sturaro D, Ruiz MA, Saboya R, et al. Addition of low-dose busulfan to cyclophosphamide in aplastic anemia patients prior to allogeneic bone marrow transplantation to reduce rejection. Bone Marrow Transplant. 2004;33(1):9-13.
24. Chen XH, Gao L, Zhang X, Gao L, Zhang C, Kong PY, et al. HLA-haploidentical blood and bone marrow transplantation with anti-thymocyte globulin: long-term comparison with HLA-identical sibling transplantation. Blood Cells Mol Dis. 2009;43(1):98-104.
25. Li JM, Giver CR, Waller EK. Graft engineering using *ex vivo* methods to limit GVHD: fludarabine treatment generates superior GVL effects in allogeneic BMT. Exp Hematol. 2006;34(7):895-904.
26. Huang XJ, Chang YJ, Zhao XY. Maintaining hyporesponsiveness and polarization potential of T cells after *in vitro* mixture of G-CSF mobilized peripheral blood grafts and G-CSF primed bone marrow grafts in different proportions. Transpl Immunol. 2007;17(3):193-7.
27. Fang J, Hu C, Hong M, Wu Q, You Y, Zhong Z, et al. Prophylactic effects of interleukin-2 receptor antagonists against graft-versus-host disease following unrelated donor peripheral blood stem cell transplantation. Biol Blood Marrow Transplant. 2012;18(5):754-62.