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Original Article

The efficacy and safety of modified immunosuppressive therapy in patients with severe aplastic anemia and transfusion-dependent non-severe aplastic anemia: a retrospective cohort study

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Abstract: This study aimed to evaluate the efficacy and safety of combined immunosuppressive therapy (IST) plus mesenchymal stromal cell infusion (MSCI) and/or umbilical cord blood infusion (UCBI) in severe aplastic anemia (SAA) or transfusion-dependent non-severe aplastic anemia (TD-NSAA) patients. A total of 79 patients with SAA or TD-NSAA were recruited as the study cohort and divided into the IST group (n=30), the IST+UCBI group (n=28), or the IST+UCBI+MSCI group (n=21) according to the treatment each patient underwent. The patients in the three groups were treated with intravenous rabbit antithymocyte globulin for 5 days and oral cyclosporine A (CsA). The patients in the IST+UCBI and IST+UCBI+MSCI groups were treated with an additional unrelated UCBI and/or MSCI. At 6 months post treatment, the overall response rate (ORR) in the IST+UCBI+MSCI group was higher compared with the ORR in the IST group (81.0% vs 50%, *P*=0.039). The IST+UCBI and IST+UCBI+MSCI groups achieved shorter median times to response (TTR) than the IST group did (84.2 days vs 145.5 days, *P*=0.010; 84.3 days vs 145.5 days, *P*=0.008, respectively). There were no significant differences in the incidences of relapse, serum sickness, Epstein-Barr virus (EBV) reactivation, cytomegalovirus (CMV) reactivation, or serious infection in the three groups. The estimated 5-year overall survival (OS) and failure-free survival (FFS) did not differ in the three groups. A logistic regression analysis revealed that IST+UCBI+MSCI and very SAA (VSAA) can predict ORR. However, only the response to IST was found to be predictive of improved OS and FFS. These data suggest that IST+UCBI+MSCI achieves a higher ORR and a shorter TTR than IST and are well tolerated in SAA and TD-NSAA patients.

Keywords: Aplastic anemia, immunosuppressive therapy, umbilical cord blood, mesenchymal stromal cells

Introduction

Aplastic anemia (AA) is a heterogeneous bone marrow failure syndrome characterized by peripheral cytopenia and bone marrow hypoplasia. Untreated, it is a potentially fatal disease, most commonly from serious infections or life-threatening hemorrhages. The incidence is 2-2.4 per million per year in Europe, but it is higher in China, affecting 7.4 patients per million [1, 2].

Once a patient is diagnosed with AA, the standard-of-care treatment is based on the patient's age, performance status, and the severity of the disease. If a patient is diagnosed with severe AA (SAA) or very severe AA (VSAA), hematopoietic stem cell transplantation (HSCT) is recommended in young and adult patients who have a matched sibling donor (MSD) [3]. Transfusion-dependent non severe AA (TDNSAA) patients are always dependent on red cells or platelet transfusions. The treatment principle of TD-NSAA should be the same for SAA if there is no response to the ciclosporin A (CsA) treatment within 6 months [4].

Immunosuppressive therapy (IST) with antithymocyte globulin (ATG) and CsA is recommended as the first line treatment for SAA patients who lack an MSD and for patients aged >50 years [3]. Little progress in IST treatment has been made in the past few decades. As we all know, the response to IST usually occurs an average of 3-6 months from the treatment [5].

The response rates are in the range of 50-70% after first-line IST [6, 7]. About 30% of patients do not respond to IST, and one third of the responders eventually relapse [8]. Horse-ATG (h-ATG) is better in terms of response rate than rabbit-ATG (r-ATG) [9-11]. Unfortunately, h-ATG is not available in China, leading us to explore new approaches to improve the outcome of IST.

Umbilical cord blood (UCB) is an alternative hematopoietic stem cell source for young patients with SAA. However, the low cell dose, the higher risk of rejection, and the delayed immune recovery limit its application [12]. Yu first reported that SAA patients who received IST combined unrelated UCB infusions exhibited higher efficiency and reduced relapse rates [13]. Then Li et al. also confirmed that IST combined with UCB infusions shortened the neutropenia durations in 30 SAA patients [14]. Mesenchymal stromal cells (MSCs) are multipotent stem cells with the capacity to differentiate into chondrocytes, adipocytes, osteoblasts, and other cells, as important stromal components of bone marrow [15]. MSCs have been widely used in HSCT to reduce the graft-versus-host disease (GvHD) and to promote the engraftment thanks to their immunomodulatory properties and the ability to support hematopoiesis [16, 17]. Some studies reported that treatment with MSCs may be a promising therapeutic strategy for patients with refractory AA. The response rates ranged from 28.4% to 33% [18, 19]. However, there are no reports on the efficacy and security of IST combined with MSC infusion.

Thus, the aim of this study was to evaluate the efficacy and safety of modified IST (MSCs and/or UCB infusion plus IST) treatment in comparison to IST treatment alone.

Methods

Patients and disease severities

We retrospectively searched the written and electronic data of 79 patients with SAA or TD-NSAA treated at the Department of Hematology, The First Affiliated Hospital of Zhejiang Chinese Medical University during the period January 2013 to December 2017. Patients with underlying inherited bone marrow failure were excluded. Patients who had clonal cytogenetic abnormalities, features of myelodysplastic syndrome, or classic paroxysmal nocturnal hemoglobinuria were also excluded.

The disease severities were defined as flows, and a diagnosis of SAA requires marrow cellularity <25% (or 25%-50% with <30% residual hematopoietic cells), plus at least 2 of the following abnormalities: neutrophil count $<0.5\times10^9$/l, platelet count $<20\times10^9$/l, and an absolute reticulocyte count $<20\times10^9$/l. VSAA meeting the criteria of SAA but with a neutrophil count $<0.2\times10^9$/l. TD-NSAA, AA not fulfilling the criteria of SAA or VSAA, but with any of the following indications for transfusion dependence: platelet count $<10\times10^9$/l, hemoglobin <60 g/L.

The patients were fully informed of the advantages and disadvantages of UCB and MSCs infusions. Informed written consent was obtained from the patients or their parents in accordance with the Declaration of Helsinki. The employed treatment of the UCB and MSC infusions was approved by the Ethics Committee of The First Affiliated Hospital of Zhejiang Chinese Medical University.

Treatments

The patients selected the IST or modified IST option according to the clinical practices and their willingness in the real world. Then the population was divided into the IST group (control group, n=30), the IST plus UCB infusion group (IST+UCBI group, n=28), or the IST plus UCB+MSCs infusion group (IST+UCBI+MSCI group, n=21) depending on the treatment option each patient received.

In the IST group, the patients were treated with intravenous r-ATG (3-4 mg/kg/day) for 5 days and oral CsA at a dose of 5-6 mg/kg/day. The CsA doses were adjusted to maintain the levels of 150-250 μg/l, and they were given for at least 1 year. In the IST+UCBI group, the patients were treated with r-ATG and CsA at the same doses and frequencies as the patients in the IST group. Then, single-unit unrelated umbilical cord blood (Shanghai Cord Blood Bank, China) was infused at day 0 after ATG. In the IST+UCBI+MSCI group, the patients were treated with r-ATG and CsA at the same doses and frequencies as the patients in the IST group. Then a single-unit unrelated umbilical cord blood was transfused at day 0 after ATG, and MSCs

derived from UCB (Shanghai Cord Blood Bank, China) infusion (Total nuclear cells, TNC, 1×10^7 cells/kg) was conducted weekly at days 7, 15, and 23.

All the patients were admitted to class 100 laminar flow clean wards after a skin-care bath. Recombinant human granulocyte colony-stimulating factor (G-CSF) was given from day 1 at a dose of 5 μg/kg/day until the neutrophil count was $\geq1.5\times10^9$/l. Red blood cell transfusion was given to those who had hemoglobin levels <60 g/l or to those who were symptomatic with hemoglobin levels <80 g/l. Platelet transfusion was given when the platelet count was $<10\times10^9$/l. In the case of bleeding, platelet counts $<20\times10^9$/l were accepted as a trigger for transfusion.

Definitions and assessments

The responses to IST were evaluated at 6 months post treatment. Complete response (CR), was defined as a neutrophil count of $>1.5\times10^9$/l, a platelet count of $>100\times10^9$/l, and a hemoglobin level of >110 g/l. Good partial response (GPR) was defined as a neutrophil count of $>1.0\times10^9$/l, a platelet count of $>50\times10^9$/l and a hemoglobin level of >110 g/l. Partial response (PR) was defined as a neutrophil count of $>0.5\times10^9$/l, a platelet count of $>20\times10^9$/l, and no transfusion dependence. No response (NR) was defined as still meeting the criteria of SAA or a dependence on red cell or platelet transfusion. Relapse was defined as a conversion to no response from a PR, GPR, or CR and/or a requirement for transfusions. Overall response rate (ORR) was defined as the rate of CR+GPR+PR. The degree of HLA match was tested prior to treatment with UCBI. Chimerism for UCBI was not tested in this study because unexpected UCB engrafts were rare [14].

We also evaluated the time to hematopoietic recovery, that is, time to response (TTR) defined as the time to achieve PR unless someone did not achieve PR at 6 months after the treatment. Furthermore, the common adverse events such as serum sickness, Epstein-Barr virus (EBV) reactivation, cytomegalovirus (CMV) reactivation and serious infection were evaluated according to the Seattle Toxicity Criteria [20]. The overall survival (OS) and failure-free survival (FFS) rates were also analyzed. OS was calculated from the day the treatment started to the time of death by any cause or the last-follow up. FFS was defined as survival with a response. Treatment failure included NR by 6 months and beyond, disease relapse, and death. The last follow-up for all the surviving patients was June 30, 2019.

Statistical analysis

The patients' baseline characteristics were compared using chi-square tests (or Fisher's exact tests if required by the sample size) for the categorical variables, and by Mann-Whitney U and Kruskal-Wallis tests for the continuous data. The OS and FFS were calculated using the Kaplan-Meier method, and the groups were compared using log-rank tests. We used a univariate logistic regression analysis to determine whether any of the selected factors were predictive of the ORR, and all factors with $P<0.1$ in the univariate analysis were estimated using the multivariate logistic regression model. A univariate Cox proportional hazard regression was used to analyze the factors affecting OS or FFS, then all the factors with $P<0.1$ were estimated using a multivariate analysis. A p value <0.05 was considered to be significant. Statistical Packages for the Social Sciences v. 17.0 (SPSS Inc., Chicago, IL) was used for the statistical analyses.

Results

Baseline characteristics

The baseline characteristics of the IST, IST+UCBI, and IST+UCBI+MSCI groups were compared as shown in **Table 1**. The median ages of the patients in the three groups were 25 years (range, 12-63), 35 years (range, 17-71), and 38 years (range, 12-26), respectively (P=0.046). There were 23 males and 7 females in the IST group, 11 males and 17 females in the IST+UCBI group, and 13 males and 8 females in the IST+UCBI+MSCI group (P=0.015). There were no significant differences in disease severity, time from diagnosis to treatment, blood counts, the degree of UCB HLA match, or UCB cell doses.

Response to treatments

First, we compared the responses to treatment at 6 months among the three groups. Five patients (2 in the IST group, 2 in the IST+UCBI

Table 1. Patient characteristics

Variable	IST group (N=30)	IST+UCBI group (N=28)	IST+UCBI+MSCI group (N=21)	*P* value
Age, median, yr (range)	25 (12-63)	35 (17-71)	38 (12-66)	0.046
Sex, n (male/female)	23/7	11/17	13/8	0.015
Disease severity, n (%)				0.690
VSAA	6 (20)	6 (21.4)	7 (33.3)	
SAA	17 (56.7)	18 (64.3)	10 (47.6)	
TD-NSAA	7 (23.3)	4 (14.3)	4 (19)	
Diagnosis to IST, m (range)	2.25 (0.8-78)	2 (0.7-19)	1.8 (0.5-228)	0.632
Median ANC, $\times10^9$/l (range)	0.3 (0-1.7)	0.34 (0-1.2)	0.36 (0.01-1.17)	0.945
Median Hb, g/l (range)	62 (32-107)	63.5 (32-132)	56 (25-78)	0.093
Median PLT, $\times10^9$/l (range)	11 (2-25)	6.5 (1-37)	10 (1-17)	0.464
Median ARC, $\times10^9$/l (range)	22.1 (4.8-44.6)	18.7 (6.7-35)	16.7 (3.4-49.9)	0.104
UCB HLA match, n (%)				0.247
4/6		6 (21.4)	1 (4.8)	
5/6		9 (32.1)	9 (42.9)	
6/6		13 (46.4)	11 (52.4)	
UCB Cell dose				
TNC (10^7/kg)		3.26 (1.98-5.7)	3.21 (1.21-6.7)	0.785
$CD34^+$ (10^5/kg)		0.51 (0.23-0.94)	0.54 (0.32-0.98)	0.347

IST: Immunosuppressive therapy; UCBI: Umbilical cord blood infusion; MSCI: Mesenchymal stromal cell infusion; VSAA: Very severe aplastic anemia; SAA: Severe aplastic anemia; TD-NSAA: Transfusion-depending none-severe aplastic anemia; ANC: Absolute neutrophil count; HB: Hemoglobin; PLT: Platelet; ARC: Absolute reticulocyte count; HLA: Human leukocyte antigen; TNC: Total nuclear cells; n: Number; yr: Year; m: Month.

Table 2. Response to treatments at 6 months

Variable	IST group (N=30)	IST+UCBI group (N=28)	IST+UCBI+MSCI group (N=21)	*P* value
CR, n (%)	1 (3.3)	4 (14.3)	3 (14.3)	0.293
GPR, n (%)	4 (13.3)	7 (25)	3 (14.3)	0.453
PR, n (%)	10 (33.3)	8 (28.6)	11 (52.4)	0.205
ORR, n (%)	15 (50)	19 (67.9)	17 (81)	0.068*
TTR, median (range)	145.5 (45-180)	84.2 (45-160)	84.3 (30-180)	0.011
Relapse rate, n (%)	5 (16.7)	3 (10.7)	3 (14.3)	0.917

IST: Immunosuppressive therapy; UCBI: Umbilical cord blood infusion; MSCI: Mesenchymal stromal cell infusion; CR: Complete response; GPR: Good partial response; PR: Partial response; TTR: Time to response; n: Number; d: Day. The differences between groups were determined using chi-square tests (or Fisher's exact test if required by the sample size). *ORR was significantly higher in the IST+UCBI+MSCI group than in the IST group (81.0% vs 50.0%, *P*=0.024), but there was no significant difference between the IST+UCBI and IST groups (67.9% vs 50.0%, *P*=0.168).

group, and 1 in the IST+UCBI+MSCI group) died within one month due to hemorrhage or serious infection, and these cases were still included in the analysis. 15 patients (50.0%; 1 in CR, 4 in GPR and 10 in PR) in IST, 19 patients (67.9%; 4 in CR, 7 in GPR and 8 in PR) in the IST+UCBI group and 17 patients (81.0%; 3 in CR, 3 in GPR and 11 in PR) in the IST+UCBI+MSCI group responded to the treatment. There were no significant differences in the CR rates, GPR rates or PR rates among the three groups, as shown in **Table 2**. The IST+UCBI+MSCI group achieved a higher ORR compared with the IST group (81.0% vs 50.0%, *P*=0.024). However, no significant difference was observed between the IST+UCBI and IST groups (67.9% vs 50.0%, *P*=0.168), or between the IST+UCBI and IST+UCBI+MSCI groups (67.9% vs 81.0%, *P*=0.304).

Table 3. Adverse events resulting from the treatments

Variable	IST group (N=30)	IST+UCBI group (N=28)	IST+UCBI+MSCI group (N=21)	*P* value
Side effects, n (%)				
serum sickness	5 (16.7)	5 (17.9)	3 (14.3)	1.000
EBV reactivation	3 (10)	2 (7.1)	3 (14.3)	0.810
CMV reactivation	2 (6.7)	1 (3.6)	1 (4.8)	1.000
Serious infection	5 (16.7)	4 (14.3)	5 (23.8)	0.705

IST: Immunosuppressive therapy; UCBI: Umbilical cord blood infusion; MSCI: Mesenchymal stromal cell infusion; EBV: Epstein-Barr virus; CMV: Cytomegalovirus; n: Number. The differences between groups were determined using chi-square tests (or Fisher's exact test if required by the sample size).

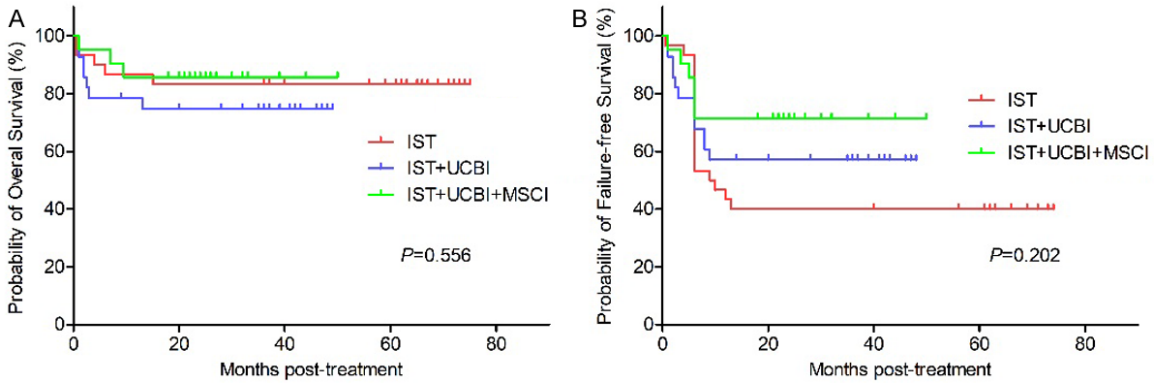

Figure 1. Overall survival and failure-free survival of the treatments. A: The estimated 5-year overall survival rates of the patients who received IST, IST+UCBI, and IST+UCBI+MSCI (83.3%±6.8%, 74.8%±8.2%, and 85.7%±7.6%, respectively; *P*=0.556). B: The estimated 5-year failure-free survival rates of the patients who received IST, IST+UCBI and IST+UCBI+MSCI (40%±8.9%, 57.1%±9.4%, and 71.4%±9.4%, respectively, *P*=0.202). *P*<0.05 was considered to be significant.

Second, we analyzed the TTR to evaluate the differences in the times to hematopoietic recovery between the three groups. Both the IST+UCBI and IST+UCBI+MSCI groups achieved shorter median TTR than the IST group did [84.2 days (range, 45-160) vs 145.5 days (range, 45-180), *P*=0.010; 84.3 days (range, 30-180) vs 145.5 days (range, 45-180), *P*=0.008, respectively] (**Table 2**). Lastly, we also evaluated the relapse rate at the last follow up time. The relapse rates in the three groups also did not significantly differ (16.7%, 10.7%, and 14.3%, respectively, *P*=0.197) (**Table 2**).

Safety profiles of the treatments

We also compared the common adverse events in the three groups at 6 months post treatment. We found that there were no significant differences in the incidences of serum sickness (16.7%, 17.9%, and 14.3%, respectively, *P*=1.000), EBV reactivation (10%, 7.1%, and 14.3%, respectively, *P*=0.810), CMV reactivation (6.7%, 3.6%, and 4.8%, respectively, *P*=1.000), and serious infection (16.7%, 14.3%, and 23.8%, respectively, *P*=0.705) (**Table 3**).

Survival analysis

The estimated five-year OS rates in the three groups were 83.3%±6.8%, 74.8%±8.2%, and 85.7%±7.6%, respectively (*P*=0.556, **Figure 1A**). The estimated five-year FFS in the three groups also did not differ (40%±8.9%, 57.1%±9.4%, and 71.4%±9.4%, respectively, *P*=0.202)] (**Figure 1B**).

Factors affecting ORR

To evaluate the possible factors affecting ORR, a univariate logistic regression was first conducted. As shown in **Table 4**, the IST+UCBI+MSCI treatment achieved a better ORR compared with the IST treatment (*P*=0.048). The patients in VSAA were likely to have a worse

Table 4. Univariate and multivariate analyses of the factors associated with ORR

Variable	ORR			
	Univariate		Multivariate	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Age (yr)	1.019 (0.989-1.051)	0.217		
Sex (Male)	0.620 (0.242-1.591)	0.320		
Disease severity				
SAA (vs. VSAA)	5.299 (1.672-16.797)	0.005	7.497 (1.438-39.082)	0.017
TD-NSAA (vs. VSAA)	4.714 (1.077-20.626)	0.039	0.920 (0.228-3.710)	0.906
Diagnosis to treatment, m	0.995 (0.974-1.016)	0.621		
ANC, $\times 10^9$/l	0.249 (0.037-1.686)	0.154		
Hb, g/l	0.993 (0.967-1.021)	0.636		
PLT, $\times 10^9$/l	0.948 (0.874-1.029)	0.203		
ARC, $\times 10^9$/l	1.015 (0.967-1.065)	0.552		
Treatment methods				
IST+UCBI (vs. IST)	1.847 (0.634-5.382)	0.261		
IST+UCBI+MSCI (vs. IST)	3.719 (1.009-13.702)	0.048	6.896 (1.469-32.380)	0.014

IST: Immunosuppressive therapy; UCBI: Umbilical cord blood infusion; MSCI: Mesenchymal stromal cells infusion; VSAA: Very severe aplastic anemia; SAA: Severe aplastic anemia; TD-NSAA: Transfusion-depending non-severe aplastic anemia; ANC: Absolute neutrophil count; HB: Hemoglobin; PLT: Platelet; ARC: Absolute reticulocyte count; ORR: Overall response rate; OR: Odds ratio; CI: Confidence interval; n: Number; yr: Year; m: Month. The factors affecting ORR were assessed using a univariate logistic regression, and the factors with a *P* value <0.1 were subsequently analyzed using a multivariate model.

ORR compared with the patients in SAA (*P*= 0.005) or TD-NSAA (*P*=0.039). Factors with a *P* value <0.1 were then analyzed using a multivariate logistic regression analysis. The results revealed that the IST+UCBI+MSCI treatment was an independent factor predicting a better ORR (*P*=0.014), and VSAA was an independent factor predicting a worse ORR (*P*=0.017).

Factors affecting survival

All the possible factors predicting survival outcomes were analyzed using a univariate cox analysis. As indicated in **Tables 5** and **6**, disease severity, ANC, and treatment efficacy significantly predicted OS, and disease severity and treatment efficacy significantly predicted FFS. Factors with a *P* value <0.1 was then analyzed using a multivariate cox regression analysis, and only the response to IST was found to be predictive of improved OS (*P*=0.002) and FFS (*P*=0.000).

Discussion

As little progress in the response to IST had been made since 1990, someone tried to modify the IST regimen to improve the efficiency. To the best of our knowledge, this is the first study to evaluate the efficacy and safety of the modified IST (IST combine with UCB and MSC infusion).

In the current study, the ORR of patients treated with IST alone was 50%, which was similar to the previous findings. Although it seemed that the IST+UCBI group achieved a higher ORR (67.9%) than the IST group, the difference was not statistically significant (*P*=0.168). However, Luo et al. reported that IST+UCBI improved the ORR from almost 57.1% to 81.8% [21]. In another study by Xie et al., IST+UCBI achieved the significantly higher ORR of 76.7% at 6 months in SAA children [22]. Our results were not consistent with these reports. This may be explained by the difference in disease severity, as we not only analyzed the VSAA/SAA patients but also included the TD-NSAA patients. The small number of cases may also influence the results. Then we found that IST+UCBI+MSCI significantly enhanced the ORR compared with IST alone (81.0% vs 50%, *P*=0.024). It is well known that the response to IST usually occurs at an average of 3-6 months [5]. However, our results showed that the TTR was shorter in the IST+UCBI+MSCI group than it was in the IST group. These results indicated that the patients who received IST+UCBI+MSCI had a higher ORR and a faster hematological recovery than the

Table 5. Univariate and multivariate analyses of the factors associated with OS

Variable	OS			
	Univariate		Multivariate	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age (yr)	1.025 (0.993-1.058)	0.125		
Sex (Male)	0.992 (0.353-2.787)	0.988		
Disease severity				
SAA (vs. VSAA)	6.581 (2.229-19.428)	0.001	2.357 (0.511-10.864)	0.272
TD-NSAA (vs. VSAA)	7.041 (0.429-23.587)	0.102		
Diagnosis to treatment, m	0.999 (0.981-1.018)	0.947		
ANC, $\times 10^9$/l	0.004 (0.000-0.149)	0.002	0.218 (0.002-27.674)	0.538
Hb, g/l	0.993 (0.963-1.024)	0.664		
PLT, $\times 10^9$/l	0.939 (0.853-1.033)	0.195		
ARC, $\times 10^9$/l	1.009 (0.960-1.061)	0.715		
Response to treatment				
Non-effect vs. effect	0.061 (0.014-0.270)	0.000	0.087 (0.019-0.405)	0.002
Treatment methods		0.567		
IST+CIBI (vs. IST)	0.627 (0.199-1.977)	0.426		
IST+UCBI+MSCI (vs. IST)	1.207 (0.288-5.052)	0.797		

IST: Immunosuppressive therapy; UCBI: Umbilical cord blood infusion; MSCI: Mesenchymal stromal cells infusion; VSAA: Very severe aplastic anemia; SAA: Severe aplastic anemia; TD-NSAA: Transfusion-dependent non-severe aplastic anemia; ANC: Absolute neutrophil count; HB: Hemoglobin; PLT: Platelet; ARC: Absolute reticulocyte count; OS: Overall survival; HR: Hazard ratio; CI: Confidence interval; n: Number; yr: Year; m: Month. The factors affecting OS were assessed using a univariate Cox proportional hazards regression, while the factors with a *P* value <0.1 were subsequently analyzed using a multivariate model.

Table 6. Univariate and multivariate analyses of the factors associated with FFS

Variable	FFS			
	Univariate		Multivariate	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age (yr)	1.015 (0.994-1.036)	0.169		
Sex (Male)	1.138 (0.589-2.196)	0.701		
Disease severity				
SAA (vs. VSAA)	3.451 (1.654-7.199)	0.001	1.311 (0.516-3.331)	0.569
TD-NSAA (vs. VSAA)	2.412 (0.969-6.005)	0.059	0.814 (0.331-2.001)	0.653
Diagnosis to treatment, m	1.000 (0.989-1.011)	0.993		
ANC, $\times 10^9$/l	0.760 (0.235-2.461)	0.647		
Hb, g/l	0.992 (0.972-1.013)	0.441		
PLT, $\times 10^9$/l	0.955 (0.900-1.013)	0.125		
ARC, $\times 10^9$/l	1.004 (0.972-1.038)	0.787		
Response to treatment				
Non-effect (vs. effect)	0.075 (0.031-0.180)	0.000	0.090 (0.037-0.222)	0.000
Treatment methods		0.567		
IST+CIBI (vs. IST)	1.324 (0.637-2.755)	0.452		
IST+UCBI+MSCI (vs. IST)	2.135 (0.845-5.396)	0.109		

IST: Immunosuppressive therapy; UCBI: Umbilical cord blood infusion; MSCI: Mesenchymal stromal cells infusion; VSAA: Very severe aplastic anemia; SAA: Severe aplastic anemia; TD-NSAA: Transfusion-dependent non-severe aplastic anemia; ANC: Absolute neutrophil count; HB: Hemoglobin; PLT: Platelet; ARC: Absolute reticulocyte count; FFS: Failure free survival; HR: Hazard ratio; CI: Confidence interval; n: Number; yr: Year; m: Month. The factors affecting FFS were assessed using a univariate Cox proportional hazards regression, while the factors with a *P* value <0.1 were subsequently analyzed using a multivariate model.

patients who received IST alone. What was responsible for this? Should we attribute this to the engraftment of the UCB cells and to the generation of transient mixed chimerisms? However, some arguments challenged the role of transient engraftment: 1) Li first reported that UCBI after high-dose cyclophosphamide/ATG resulted in a rapid hematologic recovery and a high ORR in a small sample pilot study that included 16 SAA patients. But no mixed or complete chimerism was observed in 14 of the patients [14]. Then Xie reported similar results in a larger sample of 62 SAA patients, and no patients had a donor engraftment [22]. 2) In UCB transplantation, SAA patients should receive a higher cell dose (4.9×10^7 TNC/kg and 3.9×10^5 $CD34^+$/kg) than the malignant diseases to obtain engraftment [23, 24]. The cell dose in UCBI was lower and insufficient for engraftment. 3) An intensive conditioning regimen is important to ensure engraftment, but we did not use any conditioning regimen before UCBI. 4) As everyone knows, graft failure in UCB transplantation was defined as no sign of neutrophil recovery up to 60 days post-transplantation [24]. The median TTR in the IST+UCBI+MSCI group was almost 84 days, which was longer than 60 days. We guess that many factors may enhance the effect of IST, such as follows: 1) UCB consists of abundant stem cells, the expansion of which restores the hematopoiesis. 2) UCB and MSCs bring many hematopoietic factors which can stimulate proliferation and differentiation of stem cells [25]. 3) The immunosuppressive properties of MSCs reduces immune-mediated stem cell destruction and repairs bone marrow microenvironment dysfunction. In conclusion, we attribute the better and faster hematological recovery to the hematopoietic support and immunosuppression of UCB and MSCs rather than UCB engraftment.

We also compared the complications in the three groups. There were no significant differences in the serum sickness, EBV reactivation, CMV reactivation, or serious infection among the three groups. This indicated that the modified IST treatments were safe. The relapse rate in our center was 13.9%, which was significantly lower than the 40% relapse rate reported in previous studies [26, 27]. This may be related to the not too long follow-up time in our study and to the progress of supportive care.

Unfortunately, the modified IST did not improve the OS or FFS. This may be explained by the following: 1) The total sample size of 79 patients was small, especially since these patients were divided into three groups. 2) The MSC and UCB infusions did not cause fast hematopoietic reconstruction like HSCT, which means patients face the same risk of death in the early days after treatment. In the univariate and multivariate analyses, we found that only the non-response to IST was predictive of a worse survival outcome, which is consistent with other findings [25, 28].

Conclusions

In summary, our data show that modified IST (IST+UCBI+MSCI) improved the overall response rate, shortened the time to response, and did not increase the incidences of adverse events when compared with IST. This indicates that modified IST may be an efficacious and safe regimen for SAA and TD-NSAA.

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Disclosure of conflict of interest

None.

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