

High-dose immunosuppressive therapy combined with cord blood infusion and non-myeloablative peripheral blood stem cell transplantation for patients with severe aplastic anemia

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Abstract. – OBJECTIVES: The aim of this study was to compare the clinical effective rates of high-dose immunosuppressive therapy combined with cord blood infusion (IS + CBI) and non-myeloablative peripheral blood stem cell transplantation (NSCT) for severe aplastic anemia (SAA).

PATIENTS AND METHODS: Human leukocyte antigen (HLA)-mismatched patients received immunosuppressive therapy combined with IS + CBI, whereas those with HLA matches received NSCT. Clinical effective rates, hematopoietic recovery, and prevalence of complications were compared between the two groups.

RESULTS: No significant difference in total effective rate or 2 years long-term survival was observed between the two groups. The total effective treatment in the NSCT, IS + CBI group was 80%, 68.75%, and the 2 years long-term survival rate in two groups was 2 years 76.66%, 68.75%, respectively. The median times of WBC $> 1.0 \times 10^9/L$ in the NSCT group was faster than that of IS + CBI group (13 vs 19 days) ($p = 0.027$). The median recovery times of PLT and Hb in the NSCT group was significantly faster than that of IS + CBI group (19 vs 50 days) ($p = 0.00$), (27 vs 57 days) ($p = 0.001$). The SAA group and the very SAA (VSAA) group did not show a significant difference in effective rate (76.74% vs 68.42%) ($p = 0.490$). In the NSCT group, two preparative regimens did not show a significant difference in effect (70.59% vs 92.31%) ($p = 0.141$).

CONCLUSIONS: IS + CBI is applicable to HLA-mismatched patients with SAA. NSCT is the treatment of choice for patients with HLA matching alleles. Both treatment methods are effective on VSAA.

Key Words:

Severe aplastic anemia (SAA), Immunosuppressive agents, Non-myeloablative.

Introduction

Aplastic anemia (AA) is the paradigm of human bone-marrow failure syndromes characterized by anemia, hemorrhages, and infections, which can be caused by chemical, physical and biological factors, ionizing radiation, and varied undetermined causes¹. In China, the incidence of AA shows a continuously increasing trend with the development of industry and the growth of chemicals in variety and quantity in recent years, which poses great health threat to people, particularly to the young adults. Severe AA (SAA) is a critical condition and has a rapid progression (with a course of about six months in most cases). It has a poor prognosis with very high mortality and conventional treatment cannot achieve an ideal effect on SAA^{2,3}. Nowadays, immunosuppressive agents are primarily used in pharmacotherapy for SAA⁴, although the overall survival (OS) of antihuman thymocyte globulin (ATG) combined with cyclosporine A (CsA) at 10 years lies between 60% and 80%. However, immunosuppression hematopoietic recovery is slow, the general recovery time is three to six months, and at the same time remains a suboptimal option and usually does not result in cure. About 30% of patients fail to respond, and, even in responding patients, blood counts often remain subnormal, and relapse and late clonal complications such as myelodysplastic syndromes (MDSs) are frequent^{5,6}. Therefore, allogeneic hematopoietic stem cell transplantation (Allo-HSCT) is an effective therapy for SAA at present.⁷ However, the chance to find a completely HLA (*human leukocyte antigen*)-matched donor in a family with a mean sibling number of 1.7 is only

35%⁸. This possibility turns even lower in China, in which only less than 20% of patients with SAA may have a suitable sibling donor. New treatment is important.

In our study, we perform a comparative study on the treatment effects using non-subsampled contourlet transform (NSCT) or *immunosuppressive therapy*-combined with cord blood infusion (IS + CBI) in SAA, in order to select a suitable treatment protocol for SAA patients.

Patients and Methods

Patients

A total of 62 patients with SAA received treatment in Jinan Military General Hospital between July 2002 and February 2010. All based on the Camitta's diagnostic criteria⁹. Their general data are shown in Table I. The current study was conducted in accordance with the Declaration of Helsinki and with approval from the Ethics Committee of Jinan Military General Hospital. All the patients signed a written informed consent form.

Treatment Protocols

HLA-matched patients comprised the NSCT group and the HLA-mismatched comprised the high-dose immunosuppressive therapy combined

with cord blood infusion (IS + CBI) group. The treatment protocols for different groups are shown in Figure 1.

In the NSCT group, the nucleated cell counts in HSCs ranged from $2.83 \times 10^8/\text{kg}$ to $7.8 \times 10^8/\text{kg}$ with a mean of $6.3 \times 10^8/\text{kg}$, and the CD_{34} counts ranged from $2.56 \times 10^6/\text{kg}$ to $6.98 \times 10^6/\text{kg}$ with a mean of $5.2 \times 10^6/\text{kg}$. The NSCT group was further divided into the relative and non-relative donor groups.

The relative donor group was pretreated with cyclophosphamide (CTX, $50 \text{ mg/kg} \cdot \text{d} \times 2$ (-3 d - -2 d)) and ATG ($2.5 \text{ mg/kg} \cdot \text{d}$)/ALG ($15 \text{ mg/kg} \cdot \text{d}$) ($\times 5$ (-5 d - -1 d)). Then, HSCs were infused into the peripheral blood. The nonrelative donor group was pretreated with fludarabine (FLU, $30 \text{ mg/m}^2 \cdot \text{d} \times 5$ (-5 d - -1 d)), CTX ($50 \text{ mg/kg} \cdot \text{d} \times 2$ (-3 d - -2 d)), and ATG ($2.5 \text{ mg/kg} \cdot \text{d}$)/ALG: *antilymphocyte globulin* ($15 \text{ mg/kg} \cdot \text{d}$) ($\times 5$ (-5 d - -1 d)). Then, HSCs were infused into the peripheral blood and bone marrow.

Both subgroups received preventive treatment of GVHDs (*Graft-versus-host diseases*). CsA: *cyclosporineA* ($3 \text{ mg/kg} \cdot \text{d}$) was administered intravenously from -1 d to 6 months, and the dose was then tapered slowly. Mycophenolate mofetil (MMF, 600 mg/m^2 - $1000 \text{ mg/m}^2 \times 2$ per day) was administered orally for 35 days from 0 d. Methotrexate (MTX) was given at 1 d (15 mg/m^2), 3 d (10 mg/m^2), 6 d (10 mg/m^2), and 10 d (10 mg/m^2).

Table I. The patient's basic condition.

Item	Peripheral blood non-myeloablative hematopoietic stem cell transplantation	Strong dose immunosuppressive umbilical cord blood infusion	Total
Sex, M/F	20/10	17/15	62
Median age, y (range)	26 (7-55)	14 (3-28)	
Aplastic anemia type			
SAA	18	25	43
VSAA	12	7	19
Body weight			
< 60 kg	13	23	36
> 60 kg	17	9	26
HLA locus discord			
0	25	5	30
1	3	7	10
2	2	17	19
Donor source			
Relationship	25	1	26
Sibling donor	21	1	22
Parent/child	4	0	4
No related	5	31	36
Blood type			
Match perfect	19	23	42
Main side discord	6	6	12
Minor side discord	5	3	8

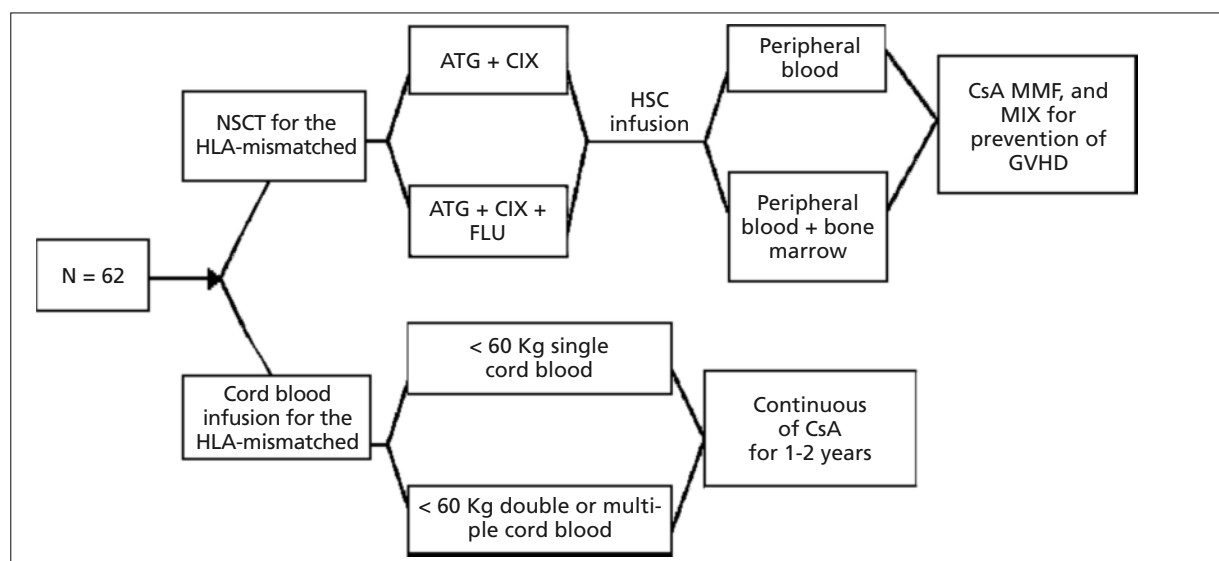


Figure 1. Different treatment protocols and preparative regimens for different groups.

IS + CBI: pretreated with CTX (50 mg/kg · d × 2 (-3 d – -2 d)) and ATG (2.5 mg/kg · d)/ALG (15 mg/kg · d) (× 5 (-8 d – -4 d)). CsA (3 mg/kg · d) was administered intravenously from -1 d to 6 months, and the dose was then tapered slowly. Single cord blood was given to patients with a TNC (*total nucleated cell*) count of 3.98-13.6 (5.97) × 10⁷/kg and a CD₃₄⁺ count of 2.67-10.5 (3.98) × 10⁵/kg, and double cord blood to those with a TNC count of 9.1-17.31 (7.2) × 10⁷/kg and a CD₃₄⁺ count of 6.75-14.2 (5.33) × 10⁵/kg.

Indexes

Therapeutic effects were evaluated according to complete remission (CR), partial remission (PR) and non-remission (NR) based on the Camitta's criteria⁹. The sum of the CR rate and the PR rate is the total effective rate. Observation indexes of hematopoietic recovery included a white blood cell (WBC) count > 1.0 × 10⁹/L and an absolute neutrophil count (ANC) > 0.5 × 10⁹/L, and a platelet (PLT) count > 20 × 10⁹/L and a hemoglobin (Hb) count > 60 g/L without concentrated red cell or PTL infusion.

Complications were also observed, including infections, hemorrhages, and organ dysfunction.

Statistical Analysis

Statistical analysis was performed with SPSS13.0 software (SPSS Inc., Chicago, IL, USA), using χ^2 -test for comparisons of enumeration data and the rates and *t*-test for those of measurement data between groups.

Results

Comparison Between Groups

The total effective rate in the NSCT group was 80%: CR (23 cases) + PR (one case)/30 cases, implantation rate was 83.33% (25/30), mortality rate was 20% (6/30), survival rate of 2 years was 76.66% (23/30). While the total effective rate in the IS + CBI group was 68.75%: CR (22 cases) + PR (0 case)/32 cases, mortality rate was 31.25% (10/32), survival rate of 2 years was 68.75% (22/32). No significant difference in CR, PR, NR or total effective rate was observed between the two groups ($p > 0.05$).

Hematopoietic Recovery

The median times of WBC > 1.0 × 10⁹/L in the NSCT group was faster than that of IS + CBI group (13 vs 19 days) ($p = 0.027$). The median recovery times of PLT and Hb in the NSCT group was significantly faster than that of IS + CBI group (19 vs 50 days) ($p = 0.00$), (27 vs 57 days) ($p = 0.001$).

Complications

No significant difference in incidence of complications was observed between the groups ($p > 0.05$, Figure 2).

Comparison Between the SAA Group and the VSAA Group

The CR rate in the SAA group was significantly higher than that in the VSAA group (32 vs 13 cases) ($p = 0.00$), but no significant differences in

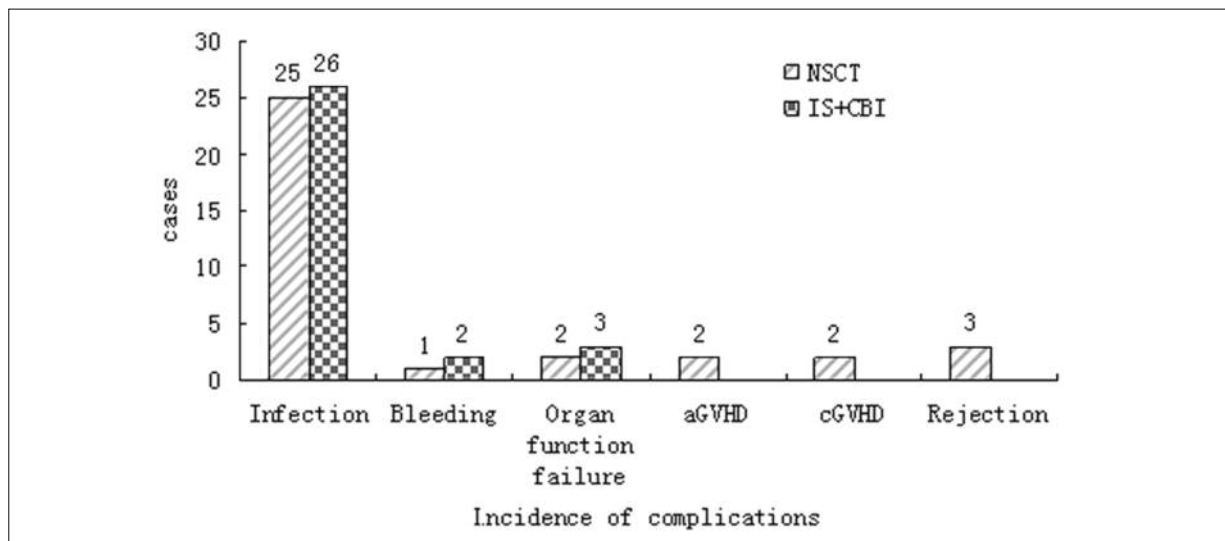


Figure 2. Prevalence of complications.

the total effective rate (76.74% vs 68.42%) ($p = 0.049$). Two different protocols did not show any significant difference in effective rates in the SAA group ($p = 0.385$), NSCT showed a significantly higher CR rate in the VSAA group ($p = 0.05$) but did not show significant differences in other indexes ($p > 0.05$) (Table II).

Comparison Between Different Preparative Regimens in NSCT

The ATG + CTX group showed a significantly higher NR rate than the ATG + CTX + FLU group (5 vs 1 cases) ($p = 0.021$), but no significant differences in other indexes were observed ($p > 0.05$) (Table III).

Discussion

Non-myeloablative allogeneic hematopoietic stem cell transplantation (NSCT) was first put

forward and then applied in clinic by Giralt et al¹⁰ in 1997. This technique significantly reduces pretreatment intensity and increases pre- and post-transplantation immune treatment intensity; so it has mild toxic side effects and lower prevalence of graft versus host diseases (GVHDs), less physical injuries and released cytokines, and lower prevalence of transplantation-related risks; and it improves patients' quality of life but at a reduced cost¹¹. In the present study, the conventional standard NSCT was further modified, based on the authors' knowledge of the pathogenesis and characteristics of SAA: The dose of CTX was only 50% of the conventional, and its effect was not to remove bone marrow excessively but to complete total implantation aided by powerful ALG/ATG combined with MMF, CsA, and FLU. In this study, most patients passed through the myelosuppression stage smoothly, and reconstituted hematopoietic function. Of the patients treated with NSCT, 23 got normally-re-

Table II. Effective rate comparison of SAA and VSAA (cases).

Diagnosis	CR	PR	NR	Total effective rate (CR+PR) (%)	Mortality rate	Survival rate of 2 years
SAA (NSCT)	14	1	3	15/18 (83.33)	4/18 (22.22)	14/18 (77.77)
SAA (IS+CBI)	18	0	7	18/25 (72.00)	7/25 (28.00)	18/25 (72.00)
<i>p</i> value	0.317	0.157	0.074	0.385		
VSAA (NSCT)	9	0	3	9/12 (75)	3/12 (25.00)	9/12 (75.00)
VSAA (IS+CBI)	4	0	3	4/7 (57.14)	3/7 (42.85)	4/7 (57.14)
<i>p</i> value	0.050*	—	1.000	0.419		0.419

* $p < 0.05$, ** $p < 0.01$.

Table III. Effective rate comparison of two pretreatment group.

Pretreatment method	CR	PR	NR	Total effective rate (CR+PR) (%)
ATG+CTX	11	1	5	12/17 (70.59)
ATG+CTX+FLU	12	0	1	12/13 (92.31)
<i>p</i>	0.768	0.157	0.021*	0.141

* $p < 0.05$, ** $p < 0.01$.

covered hemograms and long-term disease-free survival (one was partially relieved); they were not found with relapse, malignant tumor or clonal diseases; and the total effective rate reached 80%, implantation rate was 83.33%, mortality rate was 20%, and survival rate of 2 years was 76.66%. Meanwhile, reduced pretreatment intensity in this study did not result in a reduced implantation quantity: 23 patients got total implantation after hematopoietic recovery, two of whom transformed into patients with AA two years later; one showed chimera formation at 3 months, and then got total implantation through donor lymphocyte transfusion; and the implantation rate reached 83.33%. Comparison between the NSCT group and the IS + CBI group show that NSCT significantly shortens the time for three-series hematopoiesis. This result is presumably correlated with the implantation of peripheral HSCs as they are relatively mature and have a promotive effect on hematopoietic recovery.

A standard ideal donor in HSCT refers to a completely HLA (HLA-A, -B, and -DR) - matched healthy sibling. To date, nonrelative volunteer donor marrow banks have been established across the world. However, they cannot provide each patient with a non-relative HLA-identical donor¹². It will take three or four months to find a nonrelative HLA-identical volunteer donor which will be too long for a patient with SAA to wait (especially for a patient with VSAA). Therefore, to find new HSC sources has become a hotspot in this country. Nowadays, cord blood has become a new source of HSCs because of its following merit: the time for tissue matching is short; reserved cord blood can be provided at any time; it has weak antigen expression; and cord blood MSCs can perform an immunoregulatory effect and promote hematopoietic recovery. Hence, IS + CBI for patients with SAA was explored in the present study. The results showed that the patients treated with IS + CBI did not show a significant difference in total effective rate, while the total effective rate in the

IS + CBI group was 68.75%, mortality rate was 31.25%, and the survival rate of 2 years was 68.75%. The median times of WBC $> 1.0 \times 10^9/L$ in the NSCT group was faster than that of IS + CBI group. The median recovery times of PLT and Hb in the NSCT group was significantly faster than that of IS + CBI group. The time for hematopoiesis in the cord blood group was significantly slower compared with the NSCT group, but significantly faster than that of ATG combined with CsA.

In this study, the two groups did not show any significant difference in complication occurrence. In this study we also found that the total effective rate using the two different treatment protocols on the VSAA group was 68.42%, which was not significantly different from that of the SAA group (although the CR rate was significantly lower). Therefore, both NSCT and IS+CBI are effective on VSAA. They can be selected according to whether or not there is a matching donor.

According to literature, 76 patients with SAA out of 81 pretreated with ATG + CTX gained stable implantation¹³, and even those with multiple histories of blood transfusion also gained good implantation and long-term survival rates after ATG + CTX pretreatment¹⁴. In the present study, patients with SAA who received HLA-identical sibling HSC transplantation were separately pretreated with two different regimens. The results showed that no significant difference total effective rate was observed between the two groups.

Conclusions

NSCT is the first choice for HLA-matched patients with SAA, and high-dose immunosuppressive therapy combined with cord blood infusion is more suitable for the HLA-mismatched who are under a dangerous condition. Although both treatment protocols are effective on SAA-II, NSCT is the preferred one. ATG + CTX + FLU

preparative regimen is the first choice for patients who receive NSCT and nonrelative donor HSC transplantation, and ATG + CTX regimen is better to take for those who receive high-dose immunosuppressive therapy combined with cord blood infusion. As complications mainly take the form of infections, the sterility and cleanliness of the laminar air flow ward should be further improved and the preventive application of antibiotics should be intensified. In high-dose immunosuppressive therapy combined with cord blood infusion, antibiotics can be preventively used when a hematopoietic recovery delay occurs.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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