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Comparison of two dosages of rabbit antithymocyte globulin (r-ATG) in treating children with severe aplastic anemia

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In this study, efficacy and safety of two different dosages of rabbit antithymocyte globulin (r-ATG) combined with cyclosporine (CsA) in treating children with severe aplastic anemia (SAA) were compared. The clinical data of 122 SAA children treated by r-ATG/CsA between Jan 2005 and Jan 2017 at Sun Yat-sen Memorial Hospital of Sun Yat-sen University were retrospectively analyzed. The r-ATG dose of 55 cases was 2.5mg/(kg·d, group 1), and in the other 67 cases it was 3.5 mg/(kg·d, group 2). r-ATG was continuously administered for 5 days. In the 3rd and 6th month after treatment, the efficacy rate of group 2 was higher than that of group 1 (45.5% vs 26.4%, P=0.032; 54.5% vs 35.8%, P=0.042). In the 9th and 12th month after treatment, the efficacy rates of both groups were similar (71.2% vs 54.9%, P=0.077; 75.9% vs 68.6%, P=0.399). The incidence rates of serum diseases (74.5% vs 79.1%, P=0.551), short-term infection rates (76.4% vs 62.7%, P=0.105), early mortality (3.6% vs 1.5%, P=0.447), and 3-year overall survival rates (89.5% vs 90.1%, P=0.932) of both groups showed no significant differences. The r-ATG/CSA therapy was safe and effective towards SAA. The final efficacies and safety of the two r-ATG dosages were equal. However, the follow-up period in this study was relatively short, so the intergroup comparison of the long-term complications and survival rates needed to be further followed up.

1. Introduction

Aplastic anemia (AA) is a rare disorder of bone marrow blood-producing function failure and characterized by bone marrow adipogenesis, hematopoietic dysplasia (or low), or peripheral blood cell reduction (Scheinberg and Young 2012; Young et al. 2006). Allogeneic hematopoietic stem cell transplantation (allo-HSCT) and immunosuppressive therapy (IST) are the most important means in treating severe acquired aplastic anemia (SAA) (Scheinberg et al. 2006; Scheinberg and Young 2012; Young et al. 2006). Matching sibling donor (MSD)-HSCT is the preferred treatment of SAA children (Scheinberg et al. 2006; Scheinberg and Young 2012; Young et al. 2006), and the long-term survival rate could reach 80% to 90% (Gupta et al. 2010; Yoshida et al. 2014). As for the MSD-lacked SAA children, the treatment options include IST and matching unrelated donor (MUD)-HSCT (Samarasinghe et al. 2014). In the past 20 years, owing to development of MUD-HSCT, several studies reported that the efficacy of MUD-HSCT towards SAA children was equal to MSD-HSCT (Bacigalupo et al. 2000; Samarasinghe et al. 2012). However, due to relatively long MUD searching time, as well as economic factors, many SAA children still choose IST.

Antithymocyte globulin (ATG) combined with cyclosporine A (CSA) is the classic scheme of SAA-intensive immunosuppressive therapy (IST) (Samarasinghe et al. 2014; Scheinberg and Young 2012). The common types of ATG include horse-originated ATG (h-ATG) and rabbit-originated ATG (r-ATG). h-ATG had already been widely used as the first-line treatment of SAA (Kamio et al. 2011; Scheinberg et al. 2008), and r-ATG was successfully used towards SAA patients relapsed after the initial h-ATG treatment or with refractory conditions (Di Bona et al. 1999; Scheinberg et al. 2006). As for the application of r-ATG as the initial SAA treatment, several research centers had already compared its efficacy with the initial h-ATG treatment, and the results of EBMT, NIH, and Brazil institutes all showed its efficacy was worse than h-ATG (Atta et al. 2010; Kulagin et al. 2006; Marsh et al. 2012; Yoshimi et al. 2013); however, the results in Asian countries and the Cleveland Research Center (USA) showed equal efficacies (Afable et al. 2011; Jeong et al. 2014; Sakamoto et al. 2013; Shin et al. 2013).

In the past ten years, the application of h-ATG has been restricted in many countries (including China), so r-ATG became the common legitimate type of ATG. The history of r-ATG as the initial SAA treatment is not long, and different research centers used different doses of r-ATG, normally 2.3~5 mg/kg/d for five consecutive days, and the optimal dose is still not clear. This retrospective analysis compared the efficacy and safety of two different doses of r-ATG [2.5 mg/kg/d and 3.5 mg/kg/d for five consecutive days] combined with CSA in treating children with SAA, aiming to provide theoretical basis for the selection of the appropriate r-ATG dose.

2. Investigations and results

2.1. Patient characteristics

Among the 122 patients, 60 were males, and 62 were females, with a median age of 6.2 years (1-15.5 years). All the patients were diagnosed with SAA or VSAA; in group 1, one patient was diagnosed as hepatitis-correlated AA, 5 exhibited positive IgM antibody of anti- EB viral capsid antigen (VCA-IgM), and CMV-DNA of one case was increased to 1.04×10^4copies/ml; in group 2, no hepatitis-correlated AA case was found, 7 exhibited positive VCA-IgM, and CMV-DNA of one case was increased to 5.98×10^4copies/ml. For all patients IBMFS, PNH, and IRP were excluded during the initial diagnosis. The general characteristics of the two groups were shown in Table 1. There was no statistically

significant difference in age, sex distribution, disease severity, disease duration before the IST treatment, and ANC, RET, and PLT during the initial diagnosis between the two groups.

Table 1: Characteristics of patients treated with r-ATG

	Group 2.5 mg/kg/d	Group 3.5 mg/kg/d	*P*
Median age (years)	7.7 (3.7-11.4)	6.0 (4.6-8.7)	0.074
Gender (male/female)	25/30	35/32	0.456
Disease severity			0.870
SAA (n/%)	42 (76.36)	52 (77.61)	
VSAA (n/%)	13 (23.64)	15 (22.39)	
Disease duration (months)	2.00 (0.5-3.0)	1.00 (0.7-2.6)	0.437
Pre-ATG hematologic parameters, median(range)			
ANC ($\times10^9$/L)	0.30 (0.14-0.49)	0.39 (0.28-0.48)	0.047*
RET ($\times10^9$/L)	18.90 (7.20-45.40)	19.50 (8.80-40.30)	0.872
PLT ($\times10^9$/L)	12.0 (8.0-18.0)	13.0 (8.0-17.0)	0.209

SAA: severe aplastic anemia, VSAA: very severe aplastic anemia; ANC: neutrophil; RET: reticulocyte; PLT: platelet.

2.2. Treatment response

There were three cases of early death among the 122 patients that were not included into the efficacy evaluation. Eight NR cases were performed HSCT and not included into the efficacy evaluation after the transplantation. The overall efficacy rates of the r-ATG treatment in the 3rd, 6th, 9th, and 12th month of SAA were 37.0%, 46.2%, 63.6%, and 72.5%, respectively. Tables 2 and 3 compare the treatment responses between group 1 and 2. After 3-month treatment, group 1 had three cases (5.7%) of CR and 11 cases (20.7%) of PR, and the overall efficacy rate was 26.4%; group 2 had two cases (3.0%) of CR and 28 cases (42.4%) of PR, and the overall efficacy rate was 45.5%. After 6-month treatment, group 1 had five cases (9.4%) of CR and 14 cases (26.4%) of PR, and the overall efficacy rate was 35.8%; group 2 had 9 cases (13.6%) of CR and 27 cases (40.9%) of PR, and the overall efficacy rate was 54.5%. The efficacy rates of group 2 in the 3rd and 6th month were better than group 1 (P=0.032, 0.042). After 9-month treatment, among the 51 patients evaluable in group 1, 28 cases (54.9%) obtained effective responses, including 8 cases (15.7%) of CR and 20 cases (39.2%) of PR. In group 2, 11 cases (18.6%) obtained CR, 31 cases (52.6%) obtained PR, and the overall efficacy rate was 71.2%. After 12-month treatment, among the 51 patients evaluable in group 1, 35 cases (68.6%) obtained effective responses, including 13 cases (25.5%) of CR and 22 cases (43.1%) of PR; in group 2, 18 cases (31.0%) achieved CR, 26 cases (44.8%) achieved PR, and the overall efficacy rate was 75.9%. The efficacy rates of the two groups in the 9th and 12th month were similar (P=0.077, 0.399), but the proportions of PR and CR to the overall efficacy rate between the two group showed no significant difference.

Table 2: Comparison of the response rate to r-ATG between two groups

	Group 2.5 mg/kg/d	Group 3.5 mg/kg/d		*P*
3 months	14/53 (26.4%)	30/66 (45.5%)	4.573	0.032*
6 months	19/53 (35.8%)	36/66 (54.5%)	4.134	0.042*
9 months	28/51 (54.9%)	42/59 (71.2%)	3.135	0.077
12 months	35/51 (68.6%)	44/58 (75.9%)	0.712	0.399

2.3. Adverse reactions and early death

The adverse reactions of r-ATG included serum diseases, infection, and early death (Table 4). The incidence of serum diseases between the two groups had no significant difference, with the median time as the 10th day (95% CI: 5.92~9.25d) and the 9th day (95% CI: 6.95~9.65d), respectively, and the difference was not statistically significant (P=0.974). Among the 41 patients that developed serum diseases in group 1, fever occurred in 30 patients (73.2%), rash in 34 patients (82.9%), itching-accompanying in 20 patients (48.8%), and muscle and joint pain in 9 patients (22.0%); among the 53 patients with serum diseases in group 2, fever occurred in 49 patients (92.5%), rash in 46 patients (86.8%), and muscle and joint pain in 12 patients (22.6%). The symptoms in all the patients disappeared after methylprednisolone treatment.

Table 3: Proportion of CR and PR as the response rate to r-ATG

	Group 2.5 mg/kg/d	Group 3.5 mg/kg/d		*P*
3 mont hr				
CR	3 (5.7%)	2 (3.0%)	2.065	0.151
PR	11 (20.7%)	28 (42.4%)		
6 mont hr				
CR	5 (9.4%)	9 (13.6%)	0.011	0.915
PR	14 (26.4%)	27 (40.9%)		
9 mont hr				
CR	8 (15.7%)	11 (18.6%)	0.048	0.826
PR	20 (39.2%)	31 (52.6%)		
12 mont hr				
CR	13 (25.5%)	18 (31.0%)	0.932	0.334
PR	22 (43.1%)	26 (44.8%)		

CR: complete response; PR: partial response

Table 4: Comparison of the adverse events between two groups

	Group 2.5 mg/kg/d	Group 3.5 mg/kg/d	*P* value
Serum sickness	74.5% (41/55)	79.1% (53/67)	0.551
Infection	76.4% (42/55)	62.7% (42/67)	0.105
Early death	3.6% (2/55)	1.5% (1/67)	0.447

The early infection rates between the two groups showed no significant difference, with the median time of infection as the 10th day (95% CI: 8.95~17.05 d) and the 8th day (95% CI: 5.24~10.76 d); however, the infection time in group 2 was obviously earlier than that in group 1 (P=0.048). Among the 42 infection cases in group 1, in 24 cases bacterial and anaerobic cultures of peripheral blood or local secretion were taken, and the results revealed four cases of positive blood culture, namely *Klebsiella pneumoniae*, *Streptococcus pneumonia*e, *Achromobacter xylosoxidans*, and *Escherichia coli*. One case of *Pseudomonas aeruginosa* was confirmed by blood culture, sputum culture, and external ear canal secretion culture. Furthermore, one patient developed a parotid abscess, and the rest were respiratory or intestinal infections. The patient with *Pseudomonas aeruginosa* died of failed infection controlling and the patient with parotid abscess died of self-abandoning the treatment, the other 40 patients (including two cases with suspected pulmonary fungal infection) were cured. Among the 42 infection cases in group 2, in 36 bacterial and anaerobic cultures of peripheral blood were taken, and three cases were diagnosed as positive, namely with *Staphylococcus epidermidis*, *Streptococcus pneumoniae* and *Escherichia coli*. One case presented a gingivitis, one a perianal abscess, the other cases were all respiratory infections. All the patients with infections (including three cases of suspected pulmonary fungal infection) were cured, and no infection-associated death occurred.

Among 122 patients, only in two patients of group 1, early death occured. One patient developed a parotid abscess 50 days after the treatment and died on the 56th day due to treatment abandonment by the family members. The other patient had otitis media, bronchopneumonia, and hemophagocytic syndrome before the treatment, developed septicemia after the treatment, and died 34 days after the treatment due to septic shock, and multiple organ failure.

2.4. Long-term mortality, recurrence, and polyclonal diseases

The 3-year overall survival rates of group 1 and group 2 were 89.5% and 90.1%, respectively, and there was no significant difference between the two groups (P=0.932, Fig.). Group 1 had 2 cases of early death and one patient died of multiple abscesses and sepsis 14 months after failed ATG therapy, two others died of severe infection in local hospitals 18 months and 38 months after ATG therapy. Group 2 had one patient who died of sepsis and septic shock 16 months after failed ATG therapy, two others died of severe infection after 19 and 21 months, respectively. Three patients in group 1 and five patients in group 2 were re-performed HSCT within two years after failed ATG treatment.. Furthermore, 3 cases in group 1 were lost during the follow-up, and 4 cases in group 2 were not followed up for three years, so the above cases were excluded when calculating the 3-year OS.

Five patients in group 1 recurred due to discontinuation (9.1%) 2 years, 2 years, 2.58 years, 3.1 years and 9.7 years after the IST treatment, respectively, but no patient in group 2 recurred till the end of the follow-up. Among the 2 cases of clonal diseases, the one in group 1 was diagnosed as acute monocytic leukemia (AML-M5) five years after the ATG treatment and two years after the CsA withdrawal, and the other case in group 2 was diagnosed as Ewing's sarcoma 14 months after the ATG treatment.

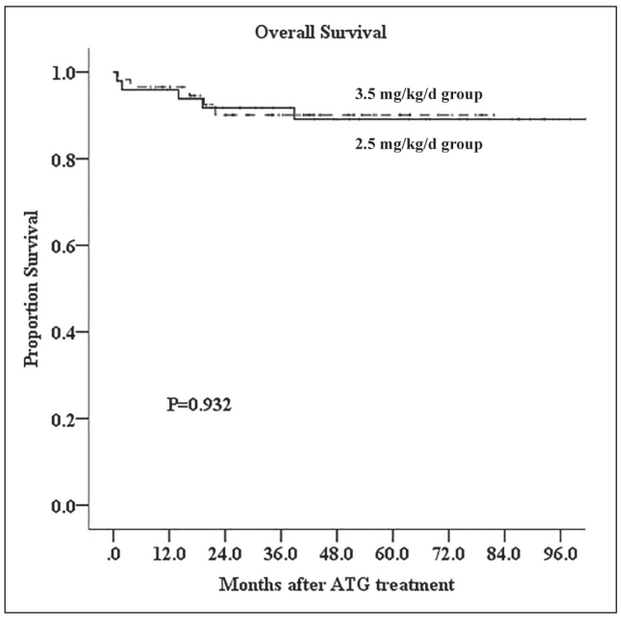

Fig: Overall survival for patients treated with different dosage of r-ATG.

3. Discussion

AA is a serious disease of bone marrow blood-producing failure, and its essence is the bone marrow hematopoietic tissue-targeted immune injury mediated by the T cell immune abnormality (Young 2013). Therefore, IST has become an important treatment method towards SAA patients where MSD-HSCT had failed. IST of h-ATG plus CSA has exhibited efficacy and safety as the first-line SAA treatment method, its treatment response rate could reach 60% to 77%, and the long-term survival rate could be more than 90% (Afable et al. 2011; Jeong et al. 2014; Kamio et al. 2011; Kulagin et al. 2006; Marsh et al. 2012; Scheinberg et al. 2008; Yoshimi et al. 2013). Previously, r-ATG has already been successfully applied to patients recurring after initial h-ATG treatment or with refractory SAA. Di Bona et al. (1999) reported response rate and survival rate of r-ATG in 30 patients with refractory SAA as 77% and 93%, respectively, which were the same as the initial h-ATG treatment. Scheinberg et al. (2008) reported the application of r-ATG in treating 22 cases of refractory SAA and 21cases of recurrent SAA, the response rate towards refractory SAA was 30%, but that towards recurrent SAA could be up to 65% (Scheinberg et al. 2008).

In recent years, r-ATG has become the only legitimate ATG type in most countries; based on the success towards refractory and recurrent SAA, many research centers have included r-ATG into the initial treatment of SAA, and compared the efficacy and safety of two different sources-derived ATG. The retrospective study of European Cooperative Group for Bone Marrow Transplantation (EBMT) showed that the 6-month response rate of r-ATG in treating 35 adult AA patients was only 40%, and the 2-year OS was significantly lower than h-ATG (68% vs 86%, P=0.009). The results of NIH (National Institutes of Health), Brazil, and Germany also showed that the efficacy and 2~3-year OS of r-ATG towards adult and pediatric SAA patients were worse than h-ATG, and the 6-month response rates of r-ATG were 37%, 34.5%, and 34%, respectively. However, recent retrospective studies of many Asian centers (including South Korea, Japan, and China) showed that the response rate of r-ATG in treating 158 SAA children was equal to h-ATG (three-month response rate: 42% vs 46%, P=0.55; six-month response rate: 55% vs 60%, P=1.0). The results in Korea and Japan also showed that these two ATG had consistent efficacies in treating adult SAA patients. This suggested that differences in the response rates of r-ATG might exist between ethnic groups. In addition, different r-ATG therapeutic dosages among different research centers might also be the reason of different therapeutic response rates.

Currently, the optimal dose of r-ATG as the first-line SAA treatment has not been determined. The previous r-ATG dose for recurrent or refractory SAA was 3.5 mg/kg/d for five consecutive days. UK treatment guidelines recommended the first-line r-ATG dose as 3.75 mg/kg/d for five consecutive days . However, different research centers used different actual doses. The doses usually used included 2.5 mg/kg/d, 3.5 mg/kg/d, or 3.75 mg/kg/d, and few centers used 5 mg/kg/d. 2.5 mg/kg/d of r-ATG was used in China, Japan, and Korea, and the 6-month response rates were 65.2%, 75%, and 45.2%, respectively. In this study, the 6-month response rate in group 1 was 35.8%, consistent with Korea while inconsistent with Japan and the previous study in our center, but the 9-month response rate was increased to 54.9%, and the 12-month response rate reached 68.6%, consistent with the previous results in this center, among who four cases of PR turned to CR, and 14 cases of NR turned to PR. Therefore, it could be seen that after 6-month treatment, some patients would still response to r-ATG. The studies of EBMT and Japan also showed that about 10~15 % of patients responded to r-ATG after 6-month treatment. European countries would prefer 3.5 mg/kg/d or 3.75 mg/kg/d, and the results of NIH and Germany showed 6-month response rates of only about 35%; the 6-month response rates of EBMT, Cleveland, and Poland were 45~50%, with an overall response rate as 60%. One Japanese study also used 3.5 mg/kg/d to treat 40 children with AA, and the 6-month response rate was 47.5%, consistent with the results of European countries and America. The 6- and 12-month response rates of group 2 in this study were 54.5% and 75.9%, respectively, and the 6-month response rates of another two studies using 5mg/kg/d in pediatric and adult patients with AA were 34.6% and 33%, respectively. These results suggested that increasing the r-ATG dose could not improve its efficacy. However, due to the impact of ethnic factors among different centers, no statistical analysis was performed, so there has been no efficacy or safety comparison among different r-ATG doses so far.

This study compared the efficacy and adverse reactions of two r-ATG treatment groups in this center. The 3- and 6-month response rates of group 2 were significantly higher than in group 1, but the two groups' 9- and 12-month response rates were equal. In addition, the incidence rates of adverse reactions between the two groups also did not show significant differences, but infections occurred significantly more often in group 2. The increasing of the r-ATG dosage should result in enhanced immunosuppression, and

this might be the cause of the earlier infection occurrence, but the infections in both groups were effectively controlled eventually, maybe owing to prophylactic therapy of antibiotics. The recurrence rate of group 1 was 9.1%, but group 2 had no recurrence case, and only one case of polyclonal diseases in each group occurred till the end of the follow-up, consistent with the results of multiple Asian centers. In this study, the follow-up period was short; therefore, more patients and longer follow-up period are needed for further observing and comparing the conditions of long-term recurrence, death, and clonal diseases.

Treatment of about 20-30% of patients might be ineffective after the initial IST treatment, and about 30% treatment responders might recur after reducing or withdrawing the drug. The 5-year FFS of MUD-HSCT in pediatric patients failed IST was up to 95%; therefore, as for the pediatric patients, remedial HSCT should have the implementation priority. ATG combining with CTX is one typical preparative regimen of HSCT towards SAA patients (Halkes et al. 2011). The patients failed IST and performed HSCT would face the re-application of ATG. Tichelli (Hutspardol et al. 2013) found in his study that the re-application of ATG in AA patients did not change the incidence rate of serum diseases; however, the incidences of 20-year late clonal diseases between the patients receiving one single course and repetitive courses of ATG showed certain difference (34±7 vs 53±10%). In addition, when used to prevent GVHD, ATG might delay immune reconstitution and cause serious infections. ATG is a risk factor of EB virus-related post-transplantation lymphoproliferative diseases (PTLD) (Tichelli et al. 1998). Among the SAA patients ever performed HSCT (Landgren et al. 2009), the incidence of EBV-PTLD in the patients received ATG-included IST was significantly higher than those who had not received ATG treatment, and the incidence of EBV-PTLD was positively correlated with the ATG courses. In the premise that the efficacies of these two doses of r-ATG were equal, considering the risks that might be brought by the re-application of ATG, 2.5 mg/kg/d × 5 d might be better selected for the initial treatment. Furthermore, during reducing the incidence of GVHD in the pretreatment, the possibilities of severe infections and EBV-PTLD should be reduced simultaneously. Therefore, how to master the suitable dosage of ATG in the pretreatment, how to cooperate simultaneously with other immunosuppressive agents so as to achieve the maximum clinical effects, are worthy of further investigation in future.

In short, IST of r-ATG combined with CsA was safe and effective for SAA. The 3-month and 6-month response rates in the 3.5 mg/kg/d group were higher than in the 2.5 mg/kg/d group, but the eventual 12-month efficacies of these two groups were equal, and the short-term adverse reactions and early mortalities of the two showed no significant differences. The follow-up period in this study was short, so the incidence of long-term complications and long-term survival rates needs to be further observed and compared to provide theoretical basis for the clinical selection of the optimal dosage of r-ATG.

4. Experimental

4.1. General data

A total of 122 children diagnosed with SAA and receiving initial treatment with r-ATG plus CsA in the Department of Pediatrics, Sun Yat-sen Memorial Hospital of Sun Yat-sen University, from January 2005 to January 2017, were included. From January 2005 to January 2010, a total of 55 patients received a 5-consecutive-day treatment of r-ATG 2.5mg/kg/d, and from March 2010 to January 2017, a total of 67 patients received 5-consecutive-day treatment of r-ATG 3.5 mg/kg/d. This study complied with the Helsinki Declaration and was approved by the Ethics Committee of Sun Yat-sen Memorial Hospital. Because all the patients were children, the informed consent was signed by their legal guardians.

4.2. Diagnostic criteria

The diagnosis and severity classification was performed in accordance with the treatment guidelines of AA recommended by the British Society of Hematology in 2009 (Marsh et al. 2012). The criteria of the severity classification were as follows: (1) SAA: the proportion of karyocytes in bone marrow was <25% or within 25%~30%, but the proportion of the residual hematopoietic cells was <30%; meanwhile, two of the following three criteria should be combined: ANC $<0.5\times10^9$/L, reticulocytes (Ret) $<20\times10^9$/L, PLT $<20\times10^9$/L; (2) very severe AA (VSAA): the criteria were the same as SAA, but ANC $<0.2\times10^9$/L; (3) non-severe AA (NSAA): did not meet the criteria of severe or very severe AA.

4.3. Treatment methods

All the patients received IST, including ATG, CsA, and methylprednisolone. ATG was r-ATG (thymoglobuline, 25 mg/ampule, Genzyme, French). Small-dose allergy test of ATG should be firstly applied (1 mg ATG was dissolved in 100 ml of saline and intravenously infused within 1 h); if severe systemic reaction or allergy occurred, this patient could not be treated with the same kind of ATG preparation; if no adverse reactions occured, ATG 2.5 mg/kg/d or 3.5 mg/kg/d could then be infused through a central venous catheter, maintaining 12-18 h once, and for 5 consecutive days; methylprednisolone was intravenously dripped before the administration of ATG, 1~2 mg/kg/d. On the fifth day of the ATG administration, methylprednisolone (methylprednisolone tablets) 1 mg/kg/d could be orally administered for nine consecutive days, and reduced the dose until stopped the administrated within five days to prevent serum diseases. During ATG administration, PLT was sampled by the infusion instrument and maintained $>30\times10^9$/L.

The dosage of CsA was 5~8 mg/kg/d, which was given in a two-time-oral-administration procedure, and the interval was 12 h. IST was started on the 14th day, and the dosage was adjusted according to the CsA plasma concentration (the whole blood trough concentration was 150~250 ng/ml before administration) and hepatonephric function. After the hemogram returned to normal and kept stable for at least six months, and the bone marrow picture was gradually remitted, CsA could then be reduced gradually.

IST treatment was performed in general wards with good air ventilation, and patients were isolated from those with infective diseases. Meanwhile, mouth care and low-bacteria diet were performed; if the patient was infected, gamma globulin (0.4~0.5 g/kg) was be infused after completing the IST treatment to enhance his/her immunity, as well as the anti-infective treatment in accordance with the criteria of agranulocytosis suggested by NCCN (National Comprehensive Cancer Network).

4.4. Efficacy evaluation

The evaluation was performed according to the criteria of Cammitta. Complete response (CR): Hb >110g/L, N $>1.5\times10^9$/L, and PLT $>150\times10^9$/L. Partial response (PR): did not rely on blood transfusion or did not reach the criteria of SAA. No response (NR): the post-treatment hemogram showed no change. Recurrence: the effects of the treatment in the treatment responders lasted at least three months, while the peripheral blood parameters decreased, and the patient needed to re-perform IST, or re-occurred the blood product transfusion dependence; the temporary reduction of peripheral blood cell count alone under normal situation or infection could not be counted as recurrence. When considering the recurrence, the patient should be performed the bone marrow examination to exclude clonal diseases. Early death: died within three months after. The efficacy evaluation was performed at least two weeks after the hematopoietic stimulating factors were stopped as well as each peripheral blood parameter was stabilized for more than four weeks. The sum of CR and PR was used to calculate the response rate, and the efficacy was assessed at the end of the 3rd, 6th, 9th, and 12th month of the r-ATG treatment, respectively. Early death was included into the analysis of adverse reactions while not performed the efficacy evaluation and analysis.

4.5. Assessment of adverse reactions

Major severe adverse reactions after the ATG treatment included: serum diseases and early infection. Early infection: referring to the infection occurred within two months after the treatment.

4.6. Follow-up

The patients performed HSCT after the IST failure were followed up until HSCT was performed. The median period of the follow-up in all the patients was 42 months (4-139 months), the median follow-up time of group 1 was 68 months (5.5-139 months), and that of group 2 was 33 months (4-82 months).

4.7. Statistical analysis

SPSS19.0 statistical software was used for the analysis. The general characteristics of all the patients were expressed as mean±standard deviation, median value, and interquartile value. The categorical data were expressed as rate or percentage. The comparison of age, disease duration, and blood parameters before the r-ATG treatment between the two groups used the rank sum test, and the comparison of gender composition, disease severity, treatment response rate, and incidence of adverse reactions between the two groups used the χ^2 test or Fisher's exact method. The survival rate estimation used the Kaplan-Meier method, and the comparison of survival curves used the Breslow test and the log-rank test, with $P<0.05$ considered as statistically significant.

Author contributions: Chun Chen, Shao-Fen Lin and Su Liu conceived and designed the study. Shao-Fen Lin, Su Liu, Hong-Man Xue, Jun-Bin Huang, Jian Wang, Qi-Hui Chen, Bi-Hong Zhang performed the experiments. Shao-Fen Lin and Su Liu wrote the paper. Shao-Fen Lin ,Su Liu and Chun Chen reviewed and edited the manuscript. All authors read and approved the manuscript.

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