OUTCOME OF SEVERE APLASTIC ANEMIA WITH DIFFERENT ABSOLUTE NEUTROPHIL COUNTS FOLLOWING IMMUNOSUPPRESSIVE THERAPY: A SINGLE-CENTER RETROSPECTIVE STUDY IN PR CHINA (2013 – 2017)

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Abstract. Treatment of severe aplastic anemia (SAA) is a challenging problem. Patients with higher absolute neutrophil counts (ANC) have better overall response rate (ORR) and longer overall survival (OS) following immunosuppressive therapy (IST). A retrospective analysis was conducted at The First Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou, Zhejiang, China between January 2013 and December 2017 involving SAA patients (n = 80) who were treated with anti-thymocyte globulin-based IST combined with cyclosporine A. Median follow-up time was 23 months. ORR at 3 months (17%) and 6 months (33%) in Group 1 patients (n = 18; ANC $<1.0\times10^8$/l) are significantly lower than Group 2 (n = 25; 1.0×10^8/l ≤ ANC $<2.0\times10^8$/l) and Group 3 (n = 37; 2.0×10^8/l ≤ ANC $<5.0\times10^8$/l) (p-value = 0.005 and 0.01 respectively). OS of Group 1 patients (34%) is significantly lower than the other two groups. Treated patients who responded at month 8 post-IST had better OS compared to non-responders (80% *vs* 44%). Univariate and multivariate analysis showed ANC is a significant factor for OS (p-values = 0.026 and 0.044 respectively). Of 24 patients who died during the follow-up period, 11 patients died of severe infection leading to septicemia in 8 patients, 7 from hemorrhage, 5 due to heart failure and 1 caused by acute myeloid leukemia. In conclusion, the study highlights absolute neutrophil counts could serve as a simple predictor of response to immunosuppressive therapy in patients with severe aplastic anemia, which should provide a guide to improved therapeutic treatment in those at high risk to succumb to fatal outcome.

Keywords: absolute neutrophil count, China, immunosuppressive therapy, severe aplastic anemia

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INTRODUCTION

Aplastic anemia (AA) is a disorder characterized by pancytopenia and bone marrow hypoplasia, which is thought to be caused by immune-mediated T cell destruction of hematopoietic stem cells (Young *et al*, 2006; Killick *et al*, 2016). Camitta *et al* (1982) proposed severe aplastic anemia (SAA) is diagnosed when a patient has marrow cellularity <25% (or 25-50% with <30% residual hematopoietic cells), together with at least two of the following criteria: absolute neutrophil counts (ANC) $<5.0\times10^8$/l, platelet counts (PLT) $<20\times10^9$/l, and corrected reticulocyte counts (CRC) <1%. Bacigalupo and co-workers further proposed very severe aplastic anemia (VSAA) be diagnosed if ANC $<2\times10^8$/l and SAA if ANC is $2.0\text{-}5.0\times10^8$/l (with criteria for and CRC and PLT remaining unchanged) (Bacigalupo *et al*, 2000).

Hematopoietic stem cell transplantation (HSCT) and immunosuppressive therapy (IST) are the main therapeutic modalities for treatment of SAA patients to improve their long-term survival with hematologic recovery, the former being treatment of choice for patients with human leucocyte antigen (HLA)-matched sibling donor (MSD) and <40 years of age (Passweg and Aljurf 2013), and the latter using anti-thymocyte globulin (ATG) combined with cyclosporine A (CsA) therapy for those without HLA-MSD and ≥ 40 years of age (Killick *et al*, 2016; Bacigalupo, 2017). A recent review (Peinemann *et al*, 2013) reported overall survival (OS) of SAA patients treated with MSD-HSCT and IST are similar, ranging 47-84% and 45-87% respectively. IST provides an alternative treatment in the situation of HLA-MSD shortage due to the one-child policy in China.

Although the association of ANC and hematologic response was well studied (Scheinberg *et al*, 2009), the role of different ANC levels in IST-treated SAA patients remains less defined; low neutrophil count is a risk factor for early mortality and OS in SAA undergoing first-line IST treatment (Atta *et al*, 2017). Here, a retrospective study was conducted on the efficacy of IST in SAA patients admitted to a hospital practicing integrated traditional Chinese and Western medicine. The findings should provide an early indicator of OS for applying appropriate ameliorating therapies.

MATERIALS AND METHODS

Study location and patient enrollment

SAA inpatients at the Department

of Hematology, The First Affiliated Hospital of University of Traditional Chinese Medicine, Hangzhou, Zhejiang, China were enrolled from January 2013 to December 2017. Exclusion criteria were (i) patients with bi-or pancytopenia from causes other than AA and (ii) patients with contraindications for IST, such as severe cardiovascular, lung, liver diseases.

Research protocols were approved by the Ethics Committee of the First Affiliated Hospital of Zhejiang Chinese Medical University (2019-x-031-01). As this was a retrospective study, no prior written consent was required but names of patients were redacted from retrieved data.

Collection of socio-demographic and laboratory data

In this retrospective study, data were collected regarding age, gender, ANC, platelet counts (PLT), reticulocytosis (Ret%), and CD4/CD8 ratio.

Treatment protocol

Diagnosis and assessment of SAA are based on criteria of Camitta *et al*, 1982. Patients were divided into three groups according to their ANC: Group 1, ANC $<1.0\times10^8$/l; Group 2, 1.0×10^8/l $\leq$ ANC $<2.0\times10^8$/l; Group 3, 2.0×10^8/l $\leq$ ANC $<5.0\times10^8$/l, and administered with 3-5 mg/kg/day ATG (Thymoglobuline, Sanofi, Paris, France) for five days, 5 mg/kg/day granulocyte colony stimulating factor (G-CSF) (Jiuyuan Genetic Engineering Co Ltd, Hangzhou, PR China) subcutaneously as required for three months to maintain ANC > 10.0×10^8/l, 5 mg/kg/day cyclosporin (CsA) (Sino-American Huadong Pharmaceutical Company, Hangzhou, PR China) for at least 12 months, and 50-75 mg/day eltrombopag (Novartis Pharma Schweiz AG, Risch-Rotkreuz, Switzerland) for at last 3 months. Supportive treatment, such as antibiotic prophylaxis and blood component transfusion, were given as required but no medication for underlying or co-morbidities during duration of IST.

Outcome assessment

Overall response rates (ORRs) [complete response (CR) + partial response (PR)] were evaluated at month 3 and month 6 post-IST, with CR if ANC $>15.0\times10^8$/l, PLT >100×109/l, hemoglobin concentration (HB) >100 g/l, and transfusion independent; and PR if ANC = $5.0\text{-}15.0\times10^8$/l, HB = 70-100 g/l, PLT >20×109/l, and transfusion independent (Killick *et al*, 2016).

Statistical analysis

Patients baseline characteristics were compared using Chi-square (or Fisher's exact test if required by sample size) for categorical variables and Mann-Whitney U and Kruskal-Wallis test for continuous variables. Kaplan-Meier method was employed for estimation of OS and comparison was based on a log-rank test. Cox regression analysis was used for univariate and multivariate analyses of factors affecting OS. A p-value <0.05 is considered significant.

Calculations were conducted with the Statistic Package for Social Science (SPSS) 25.0 software package (IBM Corporation, Armonk, NY).

RESULTS

Patients' characteristics

Patients with SAA (n = 80) enrolled at the Department of Hematology, The First Affiliated Hospital of University of Traditional Chinese Medicine, Hangzhou, Zhejiang, China, January 2013 to December 2017 were classified into three groups according to ANC as follows: Group 1 (n = 18), Group 2 (n = 25) and Group 3 (n = 37) (Table 1). There were slightly more female than male patients. The overall median (range) age was 37.5 (16-72) years. There are no significant differences in age, gender, PLT, Ret%, and CD4/CD8 ratio among the three groups (Table 1).

Response to therapy

At month 3 post-IST, CR was achieved in 11 (14%) patients, with (as expected) ORR being significantly lower in Group 1 compared to the other two groups (Table 2). At month 6 post-IST, CR increased to 17 (21%) patients, but there was no change in ORR ranking among the three groups.

Overall survival

Median (range) follow-up time was 23 (1-60) months. A Kaplan-Meir plot showed OS of 64% (n = 51) among IST-treated SAA at 60 months, with a mean ± SD estimated survival time of 42 ± 3 months (95% confident interval (CI): 37-48 months) (Fig 1A). OS was highest in Group 3 patients, followed by Group 2 then Group 1, with mean ± SD estimated survival time of 48 ± 3 months (95% CI: 41-55 months), 41 ± 5 months (95% CI: 31-51 months), and 23 ± 5 months (95% CI: 13-34 months), respectively (Fig 1B). OS of patients who had responded at month 6 post-IST is significantly higher than that of non-responders, with mean ± SD estimated survival time of 51 ± 3 months (95% CI: 45-57 months) and 31 ± 5 months (95% CI: 22-40 months) respectively (Fig 1C). Causes of death among 24 patients who died were severe infection (n =11, of which 8 developed septicemia), hemorrhage (n = 7), heart failure (n = 5), and acute myeloid leukemia (n =1).

Univariate analysis revealed baseline ANC and Ret% are significantly associated with patients OS, and only the former is an independent association factor (using multivariate analysis) (Table 3).

DISCUSSION

The study shows a significant association of baseline ANC with OS of SAA patients admitted to a hospital in China during the period 2013 to 2017. There have been previous reports of SAA patients treated with ATG-based IST that show ANC having a significant influence on short-term survival, incidence of fatal infection and a lower survival in patients with low ANC (Sleijfer *et al*, 1981).

ORRs and OS among SAA patients

Table 1

Demographic and clinical data of patients with severe aplastic anemia at the Department of Hematology, The First Affiliated Hospital of University of Traditional Chinese Medicine, Hangzhou, Zhejiang, China (January 2013 - December 2017)

Characteristic	Group 1 (n = 18)	Group 2 (n = 25)	Group 3 (n = 37)	p-value*
Median age (range), years	38 (17-68)	37 (18-61)	38 (16-72)	0.191
Gender				0.182
Male	5	12	20	
Female	13	13	17	
Clinical				
Median ANC (range), ($\times 10^8$/l)	0.3 (0-0.8)	1.3 (1.0-1.9)	3.9 (1.0-5.0)	0.000
Median PLT (range), ($\times 10^9$/l)	6 (1-8)	11 (3-20)	11 (2-25)	0.120
Median Ret% (range), (%)	0.9 (0.1-20.0)	11.1 (0.2-29.4)	11.2 (0.1-49.9)	0.692
Median CD4/CD8 ratio (range)	1.0 (0.2-3.0)	1.5 (0.7-3.0)	1.5 (0.2-4.5)	0.444

ANC: absolute neutrophil counts; PLT platelet counts; Ret%: percent reticulocytes

*Significant if p-value <0.05

Group 1: ANC < 1.0×10^8/l; Group 2: 1.0×10^8/l ≤ ANC < 2.0×10^8/l; Group 3: 2.0×10^8/l ≤ ANC < 5.0×10^8/l

Table 2

Response at month 3 and month 6 post-immunosuppressive therapy of patients with severe aplastic anemia at the Department of Hematology, The First Affiliated Hospital of University of Traditional Chinese Medicine, Hangzhou, Zhejiang, PR China (January 2013 - December 2017)

Group	Total number of patients	Number of patients at Month 3			Number of patients at Month 6		
		CR	PR	ORR	CR	PR	ORR
Group1	18	1	2	3 (17%)[a,b]	2	4	6 (33%)[c,d]
Group2	25	3	9	12 (48%)	5	10	15 (60%)
Group3	37	7	14	21 (57%)	10	14	24 (65%)
Total	80	11	25	36 (45%)	17	28	45 (56%)

CR complete response; ORR: overall response rate comprising both complete and partial responses; PR: partial response

[a]*p*-value = 0.030 compared to Group 2; [b]*p*-value = 0.005 compared to Group 3; [c]*p*-value = 0.037 compared to Group 2; [d]*p*-value = 0.010 compared to Group 3

Group 1: absolute neutrophil counts (ANC)<1.0×10^8/l; Group 2: 1.0×10^8/l ≤ ANC <2.0×10^8/l; Group 3: 2.0×10^8/l ≤ ANC <5.0×10^8/l

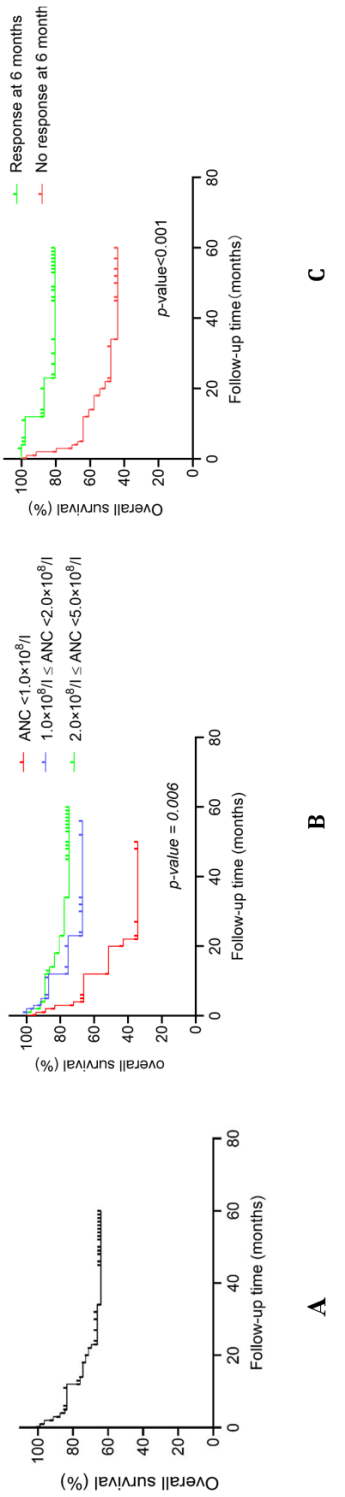

Fig 1 - Kaplan-Meyer plot of overall survival of patients with severe aplastic anemia (n = 80) undergoing immunosuppressive therapy (IST) at the Department of Hematology, The First Affiliated Hospital of University of Traditional Chinese Medicine, Hangzhou, Zhejiang, PR China (January 2013 - December 2017)

A: All patients; B: According to absolute neutrophil counts (ANC); C: According to response at month-6 post-IST

Table 3

Univariate and multivariate analyses of factors influencing overall survival of patients with severe aplastic anemia (n = 80) at the Department of Hematology, The First Affiliated Hospital of University of Traditional Chinese Medicine, Hangzhou, Zhejiang, PR China (January 2013 - December 2017)

Variable	Univariate analysis HR (95% CI)	p-value*	Multivariate analysis HR (95% CI)	p-value*
Age	1.007 (0.983-1.033)	0.557	-	-
Gender	0.535 (0.235-1.219)	0.137	-	-
ANC	0.037 (0.002-0.677)	0.026	0.07 (0.004-1.050)	0.044
PLT	0.979 (0.913-1.050)	0.551	-	-
Ret%	0.957 (0.917-0.999)	0.044	0.964 (0.923-1.007)	0.964
CD4/CD8 ratio	0.679 (0.409-1.129)	0.136	-	-

*Significant at p-value <0.05
ANC: absolute neutrophil counts; CI: confidence interval; HR: hazard index; PLT: platelet counts; Ret%: percent reticulocytes

in our study are concordant other reports. IST produced hematological recovery in 50-70% of SAA patients recruited from the Clinical Center of the National Institutes of Health in Bethesda, MD (Rosenfeld *et al*, 1995; Young and Barrett, 1995). An Asian multicenter retrospective study reported ORR of 24.3% at month 6 post-IST and the 2-year OS is 86.3 % in patients receiving rabbit ATG as first-line treatment (Chuncharunee *et al*, 2016). A prospective study by the European Blood and Marrow Transplant (EBMT) group showed ORR of up to 60% with rabbit ATG treatment and a 2-year OS of 68% (Marsh *et al*, 2012). A previous study reported 5-year OS is 86% in responders after post-IST and only 40% in non-responders (Yoon *et al*, 2012), similarly observed in our study .

Rosenfeld *et al* (2003) noted reticulocyte count as a predictive factor of long-term survival of SAA patients, suggesting this parameter may help in clinically assessing bone marrow function. A higher reticulocyte and absolute lymphocyte counts are predictive of 5-year OS reaching as high as 92% (Scheinberg *et al*, 2009). In addition, a large retrospective European study confirmed a significant link between age and prognosis, younger SAA patients having a more favorable OS (Locasciulli *et al*, 2007), and in another study younger age, ANC

$<2.0\times10^8$/l before IST, absence of invasive fungal infection, and use of voriconazole are independent variables associated with OS (Valdez *et al*, 2011). However, in our study ANC was the only independent parameter and Ret% was additionally revealed as an association factor, although both parameters showed weak statistical significance (Table 3).

Infection was a common complication associated with neutropenia and a common cause of death in our study. Haploidentical bone marrow transplantation (BMT) for patients with refractory SAA results in neutrophil engraftment in 94% of patients and a 1-year OS of 67.1% (Esteves *et al*, 2015), suggesting treatment to reduce granulocytosis may be an appropriate choice for high risk patients, especially where there is a possibility of neutrophil recovery, but the treatment has little effect on improvement in OS (Jeng *et al*, 2005; Bacigalupo *et al*, 1995; Bacigalupo *et al*, 2000). Supportive granulocyte transfusion is another option but not frequently applied because of risk of adverse side effects (Quillen *et al*, 2009). Treatment with eltrombopag shows an OS of 40% at month 4, particularly in patients with refractory aplastic anemia (Desmond *et al*, 2014). Androgens and danazol are alternative choices in patients unsuitable for BMT (Jaime-Perez *et al*, 2011).

In our study, one patient with ANC $<1.0\times10^8$/l (Group 1) who responded at month 6 post-IST evolved to AML. Several studies reported prevalence of clonal evolution to MDS/AML of 2.5-10.9% at year 10 post-IST (Socie *et al*, 2007, Li *et al*, 2011). Possible reasons of clonal evolution are differences in patient populations and treatment protocols.

Limitations of this study were its retrospective design and short follow-up time. Extended follow-up time will be needed to observe outcomes of patients with 1.0×10^8/l ≤ ANC $<5.0\times10^8$ /l (Groups 2 and 3).

In conclusion, our retrospective study reveals ANC as a predictive factor for efficacy and survival of SAA patients treated with IST. Appropriate therapeutic strategies are urgently needed for treatment of high-risk SAA patients.

ACKNOWLEDGEMENTS

The research was supported by the Natural Science Foundation of Zhejiang Province (LY19H290003), the Foundation of Zhejiang Province Chinese Medicine Science and Technology Plans (2020ZA044,2017ZB030), Zhejiang Provincial Medical and Health Science and Technology Project (2020KY196, 2018245262), and the 2017 School Research Fund Key Project of Zhejiang Chinese Medical University (2017ZZ02).

CONFLICTS OF INTEREST DISCLOSURE

The authors declare no conflicts of interest.

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