Original Article

Therapeutic Response to Immunosuppressive Agents among Thai Patients with Transplant-Ineligible Aplastic Anemia: Possible Predictive Factors

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Abstract:

Objectives: *Response to immunosuppressive therapy with its predictive factors suggests the pathogenesis of aplastic anemia differs among ethnic groups. The aims of this study were a) to investigate the therapeutic response to antithymocyte globulin (ATG)-based immunosuppressive therapy (IST) and b) to identify predictors of the response among Thai patients with aplastic anemia.* ***Materials and Methods:*** *We enrolled and reviewed medical records of patients with aplastic anemia treated and followed up at the Division of Hematology of our tertiary care hospital from April 1989-June 2012. Detection of the paroxysmal nocturnal hemoglobinuria (PNH) clone was performed using high-resolution 2-color flow cytometric analysis.* ***Results:*** *In all, 121 patients were recruited. Three with no data of definite therapy were excluded. Sixty-one patients, with severe aplastic anemia (SAA) or non-severe aplastic anemia (NSAA) who required regular transfusion support, were treated with immunosuppressive agents (IST), either ATG alone or ATG with cyclosporine A (CsA). The 6-month overall response rates (ORRs) after the first and second courses of IST were 45.2% and 53.3%, respectively. The ORRs to rabbit-ATG and horse-ATG were comparable. With multivariate analysis, lymphocyte count of more than 1,500/μL was a good predictor of response to IST (OR 3.17, 95%CI: 1.01-9.98, p = 0.048). The presence of the PNH clone of red blood cells or neutrophils did not predict response to IST.* ***Conclusion:*** *Our study demonstrated therapeutic benefits of ATG-based IST among Thai patients with transplant-ineligible severe aplastic anemia. Lymphocyte count was a good predicting factor of response to IST.*

Keywords : ● *Severe aplastic anemia* ● *Immunosuppressive therapy* ● *Antithymocyte globulin*

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นิพนธ์ต้นฉบับ

การตอบสนองต่อการรักษาด้วยยากดอิมมูนในผู้ป่วยโรคไขกระดูกฝ่อที่ไม่สามารถรักษาด้วยการปลูกถ่ายไขกระดูก: ปัจจัยทำนายการตอบสนองที่เป็นไปได้

พศวีร์ เขมะพิพัฒน์ อัญชลี เทศสวัสดิ์ เจน เจียรธนะกานนท์ อรทัย ทะก่ำ และ วันชัย วนะชิวนาวิน
สาขาวิชาโลหิตวิทยา ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

บทคัดย่อ

วัตถุประสงค์ *เพื่อศึกษา ก) การตอบสนองต่อการรักษาด้วยยากดอิมมูน (immunosuppressive therapy, IST) ที่มี antithymocyte globulin (ATG) เป็นองค์ประกอบหลัก (ATG-based IST) ข) ปัจจัยที่ทำนายการตอบสนองต่อการรักษาดังกล่าวในผู้ป่วยโรคไขกระดูกฝ่อ* ***วิธีการศึกษา*** *ทบทวนรายงานผู้ป่วยโรคไขกระดูกฝ่อซึ่งติดตามการรักษาที่สาขาวิชาโลหิตวิทยา โรงพยาบาลศิริราช ระหว่างเดือนเมษายน พ.ศ 2532 ถึง เดือนมิถุนายน พ.ศ 2555 ตรวจหา PNH clone โดย high-resolution 2-color flow cytometric analysis* ***ผลการศึกษา*** *จากผู้ป่วย 121 ราย ในช่วงเวลาดังกล่าว ผู้ป่วย 3 ราย ถูกตัดออกเนื่องจากไม่มีข้อมูลของการรักษาที่ชัดเจน มีผู้ป่วย 61 ราย ที่เข้าเกณฑ์โรคไขกระดูกฝ่อชนิดรุนแรง (severe aplastic anemia) หรือไม่เข้าเกณฑ์ชนิดรุนแรงแต่ต้องรับเลือดเป็นประจำที่ได้รับการรักษาด้วยยากดอิมมูน ATG อย่างเดียวหรือ ATG ร่วมกับ cyclosporine A (CsA) พบว่า อัตราการตอบสนองต่อการรักษาดังกล่าวที่ 6 เดือน ในการรักษาครั้งแรก (first course) และครั้งที่สอง (second course) สำหรับผู้ป่วยที่ไม่ตอบสนองต่อการรักษาครั้งแรก เท่ากับร้อยละ 45.2 และร้อยละ 53.3 ตามลำดับ โดยไม่พบความแตกต่างในการตอบสนองระหว่าง horse-ATG และ rabbit-ATG และจาก multivariate analysis พบว่าจำนวนนับลิมโฟไซต์ (lymphocyte count) ที่มากกว่า 1,500/μL เป็นปัจจัยที่ทำนายการตอบสนองต่อ IST (OR 3.17, 95%CI: 1.01-9.98, p = 0.048) การพบ PNH clone ของเม็ดเลือดแดงหรือเม็ดเลือดขาวไม่ทำนายการตอบสนองต่อ IST* ***สรุป*** *การศึกษานี้แสดงประโยชน์ของการรักษาด้วยยากดอิมมูนที่มี ATG ในผู้ป่วยไทยซึ่งเป็นโรคไขกระดูกฝ่อรุนแรงที่ไม่สามารถรักษาด้วยการปลูกถ่ายไขกระดูก และพบว่าจำนวนนับลิมโฟไซต์เป็นปัจจัยที่ทำนายการตอบสนองต่อ IST*

คำสำคัญ : ● *Severe aplastic anemia* ● *Immunosuppressive therapy* ● *Antithymocyte globulin*
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Introduction

Aplastic anemia (AA) is a hematopoietic stem cell disorder characterized by pancytopenia and hypocellular bone marrow. Its incidence was shown to be higher in Thailand than in Europe and North America, and was estimated to total 3.9-5 cases/million/year.[1-3] The disease is categorized in very severe, severe, and nonsevere groups according to peripheral blood count and bone marrow features.[4,5] Before curative treatments with either hematopoietic stem cell transplantation (HSCT) or immunosuppressive therapy (IST), most patients with severe aplastic anemia died from neutropenia related infections and thrombocytopenia-related bleeding complications.[6] Life expectancy among patients with severe disease with no curative treatment is usually shorter than 12 months.[7,8]

Both laboratory evidence and therapeutic response to immunosuppression point out that the pathogenesis of idiopathic aplastic anemia is immune-mediated in which cytotoxic T-lymphocytes and cytokines play a role in the suppression of hematopoiesis.[9] Anti-thymocyte globulin (ATG) combined with cyclosporine has been shown to be the most effective immunosuppressive regimen to treat aplastic anemia. ATG has various immunomodulatory effects including T-lymphocyte depletion in peripheral blood and lymphoid tissues, with modulation of T-cell mediated bone marrow injury.[10] In addition, clones of blood cells with paroxysmal nocturnal hemoglobinuria (PNH), a phenotype characterized by a deficiency of *glycosylphosphatidylinositol anchored protein (GPI-APs) on cell membranes,* were detected in up to 60% of patients with aplastic anemia.[11-13] *Hematopoietic cells with loss of GPI-AP are less likely to be damaged by cytotoxic T-lymphocytes. The presence of PNH clones may be a marker of immune-mediated bone-marrow failure.*[14,15] *The PNH negative clone had been shown to be a predicting factor of response to IST in patients with aplastic anemia.*[13] *Absolute reticulocyte count > 25,000/μL and absolute lymphocyte count > 1,000/μL were among other reported predictors of response to IST.*[16]

Adding cyclosporine A (CsA) to antithymocyte globulin (ATG) was shown to increase response rate from 31 to 65% *in patients with severe aplastic anemia (SAA).*[17] *This* combination has been used as a standard treatment for transplant ineligible patients. Until recently, CsA was not covered in the Thai national universal health coverage; therefore, a proportion of Thai patients with SAA were formerly treated with ATG alone. In this study, we retrospectively analyzed the therapeutic response of ATG-based IST, altogether of either ATG alone or ATG with CsA among Thai patients with transplant-ineligible, severe aplastic anemia. Predictive factors of response to ISTs were also determined, together with overall survival of the patients.

Materials and Methods

Patients & Eligibility

In this retrospective observational study, we reviewed medical records of patients with aplastic anemia of various severities, aged between 12 to 85 years old, treated and followed at the Division of Hematology of our tertiary care hospital, Bangkok, Thailand from April 1989 to June 2012. We excluded vulnerable patients with either pregnancy, CrCl < 30 mL/min, AST or ALT > threefold of normal limits, cirrhosis, cancer, heart disease or cerebrovascular disease. For our study, SAA was defined as a bone marrow cellularity of less than 25% together with two of the following blood count criteria: i) corrected reticulocyte count < 1%, ii) absolute neutrophil count (ANC) < 500/μL, and iii) platelet count < 20,000/μL. Patients who met SAA criteria and having ANC of < 200/μL were classified as having very severe aplastic anemia (VSAA).[4,5] This study was carried out after being approved by the faculty institutional review board.

Screening of the PNH Clone

Screening of the PNH clone was performed by high-resolution 2-color flow cytometric analysis according to the previously described method.[18] Presence of 0.003%

and 0.005% of deficient GPI-AP- (CD55 and CD59) granulocytes and erythrocytes, respectively in peripheral blood indicated the presence of the PNH clone.

Data Collection

Age at diagnosis, sex, disease severity, time to treatment, complete blood count, reticulocyte count, type of immunosuppressive therapy and response were collected and analyzed.

Definition of Response

Response to either immunosuppressive therapy or anabolic hormones was assessed after four to six months of treatment, confirmed by two blood counts as a minimum of four weeks apart. It was categorized in two groups; partial response (PR) defined as transfusion independence and no longer meeting criteria for severe disease and complete response (CR) defined as hemoglobin concentration $\geq$ 12 g/dL, neutrophil count > 1,500/μL and platelet count > 150,000/μL.[19]

Statistical Analysis

The statistical analysis was performed using SPSS, version 16. Numerical data were presented as frequency and percentage. Categorical data with normal distribution were presented with means and standard deviations, while those without normal distribution were presented as median, minimum and maximum.

Comparison between groups such as response rates were analyzed by Chi-square or Fisher's exact test. Overall survival was shown with percentage and 95% confidence interval. Predictors of response were analyzed by Chi-square or Fisher's exact test and independent t-test or Mann-Whitney U test. The factors with $p < 0.2$ were calculated with multiple logistic regressions and presented with adjusted odd ratio (OR) and 95% confidence interval (CI). All comparisons were prepared by two-tailed test.

Results

Patients' Characteristics

A total of 121 patients with aplastic anemia were initially recruited. They comprised 13 VSAA, 70 SAA and 38 nonsevere (NSAA) patients. Three were excluded due to lack of certain treatment data. Sixty-five patients were treated with IST and 53 patients were given only anabolic hormones.

The IST used in this study was horse ATG (Lymphoglobuline^{R}, Genzyme, USA) at a dose of 15 mg/kg/day (21 patients) or rabbit ATG, i.e., Thymoglobulin^{R} (Genzyme, USA) 2.5-3.75 mg/kg/day (33 patients) or ATG-Frasenius^{R} (Frasenius Biotech, Germany) 5 mg/kg/day (7 patients), intravenously for five days. A proportion of patients also received cyclosporine A (CsA), starting at a dose of 5 mg/kg/day after ATG, and adjusted dosage to maintain a proper serum trough level (200-300 ng/mL).

Characteristics of patients treated with IST are shown in Table 1. They comprised 32 females (49.2%) and 33 males (50.8%), median age of 37 years (range 13-81 years). Clinical severities included 11 NSAA (16.9%), 45 SAA (69.2%), and 9 VSAA (13.9%). The most common presented symptom was anemia accounting for 80% of cases. Bleeding and fever were found in 69.2 and 21.5% of patients, respectively. The baseline hematologic parameters included hemoglobin of 6.35±1.53 g/dL, reticulocyte count of 0.6% (range 0.03-2.00), platelet count of 8,000/μL (range 2,000-64,000), absolute neutrophil count of 612/μL (range 37-2,544) and absolute lymphocyte count of 1,550/μL (range 463-4,830). Detection of PNH clone was carried out in 45 patients. The median clone size for PNH granulocytes was 0.086% (range 0.000-8.158%) and for erythrocytes was 0.000% (range 0.000-2.169%). A positive PNH clone, as indicated by the presence of $\geq$ 0.003% and/or $\geq$ 0.005% of deficient GPI-AP granulocytes and erythrocytes, respectively, was found among 30 patients (66.7%).

Hematologic Response to IST

Among the 65 patients, 61 received either ATG alone (42 patients) or ATG with CsA (19 patients) as initial treatment. Four patients were treated with CsA alone. The overall response rate (ORR) to ATG was 42.6% (Figure 1). Nearly all patients (25 of 26), responding

Table 1 Characteristics of patients treated with immunosuppressive agents

Female, n (%)	32 (49.2)
Age in year, median (min-max)	37 (13-81)
Clinical diagnosis, n (%)	
NSAA (required transfusion)	11 (16.9)
SAA	45 (69.2)
VSAA	9 (13.8)
Presenting symptom, n (%)	
Anemic symptom	52 (80)
Bleeding	45 (69.2)
Fever	14 (21.5)
Hb (g/dL), mean ± SD	6.35 ± 1.53
MCV (fL), median (min-max)	89 (72-117)
Reticulocyte count (%), median (min-max)	0.6 (0.03-2.00)
Platelet (/μL), median (min-max)	8,000 (2,000-64,000)
Absolute neutrophil count (/μL), median (min-max)	612 (37-2,544)
Absolute lymphocyte count (/μL), median (min-max)	1,550 (463-4,830)
PNH -ve RBC clone (%), median (min-max)	0.000 (0.000-2.169)
PNH -ve PMN clone (%), median (min-max)	0.086 (0.000-8.158)
Time to treatment (weeks), median (min-max)	14 (2-172)

n, number; NSAA, non-severe aplastic anemia; SAA, severe aplastic anemia; VSAA, very severe aplastic anemia; SD, standard deviation; PNH, paroxysmal nocturnal hemoglobinuria; RBC, red blood cell; PMN, polymorphonuclear leukocytes; PNH -ve RBC clone normal < 0.005%, PNH -ve PMN clone normal < 0.003%

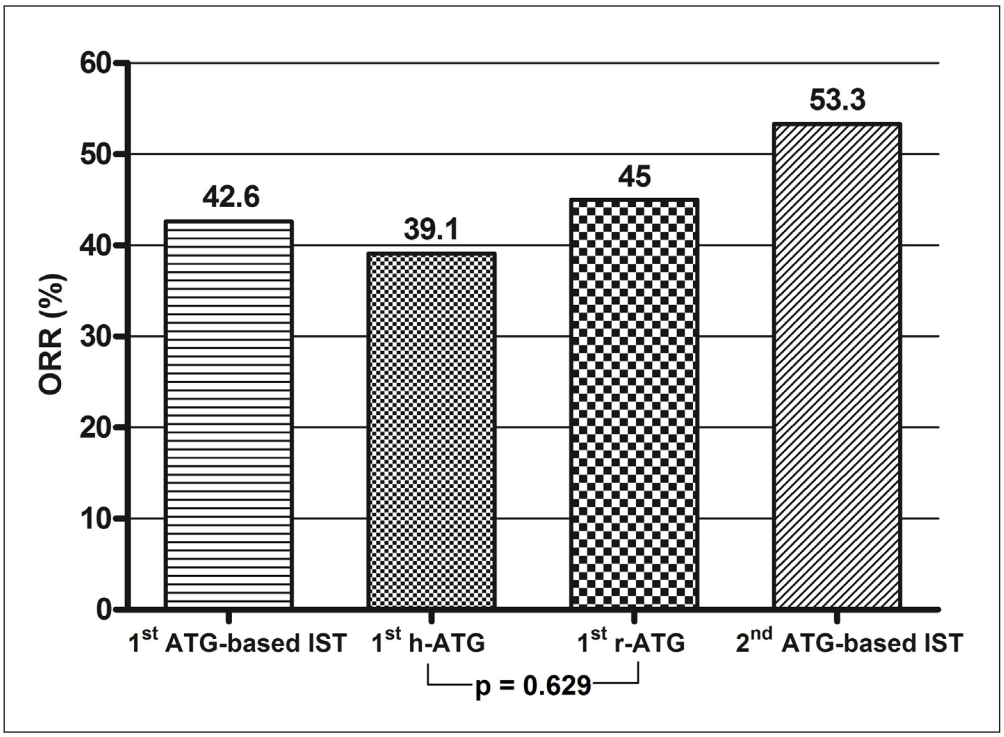

Figure 1 Overall response rate to immunosuppressive therapy (IST) at six months according to various treatment setting; 1st ATG-based IST, 1st h (horse) ATG, 1st r (rabbit) ATG, 2nd ATG-based IST

to the ATG-based IST, had remission after four months of treatment initiation. Of those who responded to ATG, CR and PR were achieved in 5 (8.2%) and 21 patients (34.4%), respectively. Regarding to the PR, 12 of the 21 responding patients (57%) improved all three hematopoietic lineages and all responding patients except one had hemoglobin increments leading to transfusion independence. No difference was observed in overall remission between those treated with horse-ATG (21 patients) and rabbit-ATG (40 patients) (ORR 39.1% vs. 45% respectively, p = 0.629). Of the four patients treated with only CsA, two achieved remission (one with CR, the other with PR) and two did not respond to the treatment.

Eighteen patients had a second course of IST, 15 were refractory to and three relapsed after the first course of IST. In the refractory group, the response rate was 53.33% (8 of 15 patients) with a CR of 20% (3 of 15 patients). Among the relapsed patients, re-treatment with IST induced a remission rate of 33.33% (1 of 3 patients).

Predictive Factors of Response to IST

Patients' characteristics including clinical features, such as age, sex, anemic or bleeding symptoms and onset of the disease before therapy did not differ in the responsive and nonresponsive groups. Among the hematologic data, lymphocyte count ≥ 1,500/μL and reticulocyte count > 0.5% favored response to IST (p = 0.023 and 0.04, respectively) (Table 2). However, with multivariate analysis, lymphocyte count > 1,500/μL was the only independent factor that predicted response to IST (OR 3.17, 95%CI: 1.01-9.98, p = 0.048). Presence of the PNH clone, either of RBC or PMN, had no predictive value of response to IST among patients with severe aplastic anemia.

Cumulative Overall Survival

Cumulative overall survival rates of patients with NSAA, SAA and VSAA at 12 and 24 months were similar at 0.9, 0.77, and 0.5, respectively. At 60 months, the cumulative survival rates of NSAA, SAA and VSAA were 0.9, 0.65, and 0.5, respectively (Table 3 and Figure 2).

Discussion

In the absence of histo-compatible donor or the presence of comorbidities, ATG-based IST is recommended as the front-line treatment of severe aplastic anemia. Horse ATG (h-ATG) had been widely used until recently, because of its limited availability, and has been replaced by rabbit ATG (r-ATG) at least in many countries including Thailand. After it was reported that adding CsA increased the response rate to ATG, the combination of ATG and CsA has become a standard front-line treatment of aplastic anemia.[17] We found that the ORR (CR+PR) to ATG-based IST in our patients was 42.6%. No significant difference was found in ORRs after h-ATG and r- ATG. Our finding of comparable efficacy between r-ATG and h-ATG is similar to some other studies.[20, 21] After six months of treatment, the ORRs of our patients to h-ATG and r-ATG were 39.1% versus 45%, which were comparable to the 39.1% versus 45.3%, respectively, from a study by Shin et al.[20] In an Asian retrospective study, the 6- and 12-month ORRs after r-ATG were 24.3 and 68.6%, respectively, and the 12-month ORR was comparable to historical results obtained with h-ATG.[22] However, these findings seemed to be discordant with other reports showing a higher overall response to h-ATG than r-ATG; 59.5% versus 34.5%[23] and 68% versus 37%.[24] There had been no difference in response to h-ATG and r-ATG in a study of European patients.[25] Apart from heterogeneity of study components, the difference in response to ATG-based IST among reported studies may reflect the disparate pathogenesis of aplastic anemia among patient populations. Epidemiological studies of aplastic anemia in Thailand have demonstrated a significant association of aplastic anemia with agricultural pesticides, farming with exposure to ducks and geese and drinking water from local sources in rural areas.[2] Therefore, the pathogenesis of aplastic anemia in Thai patients is possibly heterogeneous and immune mediated bone marrow injury may play a lesser role, resulting in a lower response to IST, as compared with patients from western countries.

Table 2 Possible predictive factors of response to IST

Factor	Response to IST		p-value
	No response, n (%)	Response, n (%)	
Sex			
Male	18 (54.5)	15 (45.5)	0.694
Female	19 (59.4)	13 (40.6)	
Mean age (years) (mean ± SD)	44.05 ± 17.68	38.25 ± 18.90	0.208
Clinical diagnosis			
NSAA	6 (54.5)	5 (45.5)	0.861
SAA	25 (55.6)	20 (44.4)	
VSAA	6 (66.7)	3 (33.3)	
Anemic symptom			
No	7 (58.3)	5 (41.7)	0.872
Yes	29 (55.8)	23 (44.2)	
Bleeding symptom			
No	13 (68.4)	6 (31.6)	0.202
Yes	23 (51.1)	22 (48.9)	
Fever			
No	27 (54.0)	23 (46.0)	0.493
Yes	9 (64.3)	5 (35.7)	
Onset to treatment (weeks)	16 (2-156)	13 (3-172)	0.686
	Median (min-max)	Median (min-max)	
Hb (g/dL) (mean ± SD)	6.19 ± 1.55	6.56 ± 1.52	0.415
Reticulocyte count (%)			
<1	15 (53.6)	13 (46.4)	0.423
≥ 1	3 (37.5)	5 (62.5)	
Reticulocyte count (%)			
< 0.5	10(71.4)	4(28.6)	0.040
≥ 0.5	8(36.4)	14(63.6)	
Neutrophil count (/μL)			
< 200	6(75.0)	2(25.0)	0.269
≥ 200	27(50.9)	26(49.1)	
Lymphocyte count (/μL)			
< 1,500	19 (70.4)	8 (29.6)	0.023
≥ 1,500	14 (41.2)	20 (58.8)	
Platelet (/μL)			
< 10,000	20 (58.8)	14 (41.2)	0.572
≥ 10,000	15 (51.7)	14 (48.3)	
PNH (GPI-ve) RBC clone			
Absence	10(43.5)	13(56.5)	0.420
Presence	7(31.8)	15(68.2)	
PNH (GPI-ve) PMN clone			
Absence	5(33.3)	10(66.7)	0.664
Presence	12(40.0)	18(60.0)	

Table 3 Cumulative survival of aplastic anemia patients treated with immunosuppressive agents

Cumulative survival	12 months	24 months	60 months
NSAA	0.9	0.9	0.9
SAA	0.77	0.77	0.65
VSAA	0.5	0.5	0.5

NSAA, non-severe aplastic anemia; SAA, severe aplastic anemia; VSAA, very severe aplastic anemia

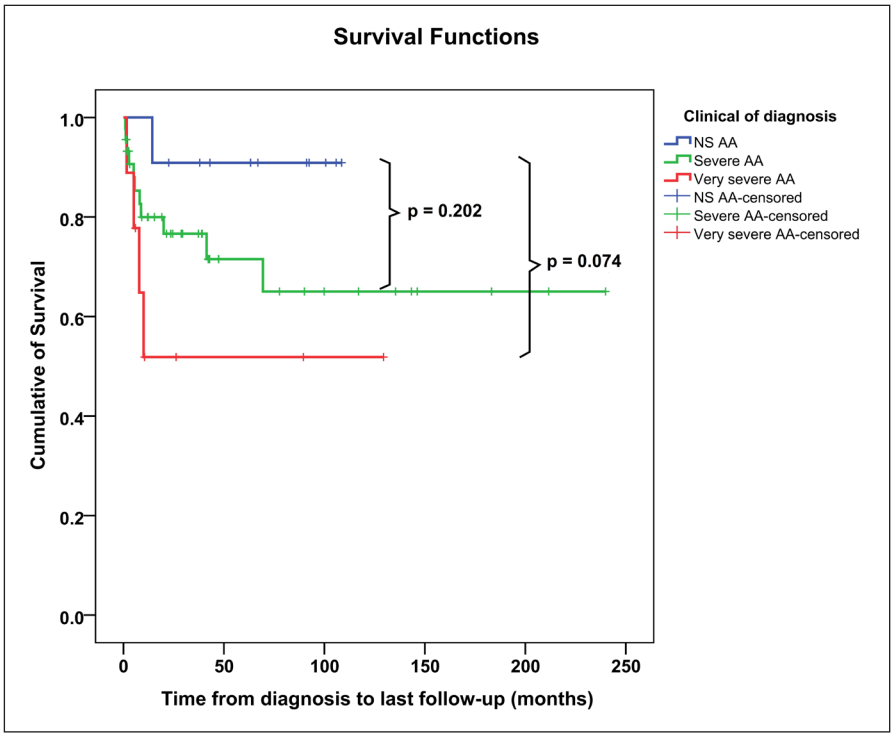

Figure 2 Kaplan-Meier curve for cumulative survival in patients with aplastic anemia treated with immunosuppressive agents; NSAA, nonsevere aplastic anemia; severe AA, severe aplastic anemia; very severe AA, very severe aplastic anemia

In our study, we assessed the long term outcomes of IST by investigating the cumulative overall survival of the patients. As expected, early mortalities were remarkable among patients with SAA and especially VSAA. After one year, the cumulative survivals between patients with SAA including VSAA and NSAA did not significantly differ, as those survived early complications of the disease and IST acquired the responsive benefits of the treatment.

Using univariate analysis, we found a lymphocyte count ≥ 1,500/μL and reticulocyte count > 0.5% favored response to IST at six months evaluation (Table 2). However, in the multivariate analysis, a lymphocyte counts ≥ 1,500/μL was the only independent predicting factor of ORR to the treatment. A lymphocyte count ≥ 1,000/μL was shown to be a good predictor of response to IST at six months in North American patients.[16] In this study, we did not find any relationship between the presence of a PNH clone and ORR to IST. Studies of predictive responses to IST of the presence of PNH clones in SAA have yielded controversial results, either favorable[26] or no responsive effect.[27] As PNH phenotype and the presence of certain HLA alleles especially HLA-DR 15 in aplastic anemia suggest its immune mediated pathogenesis,[13,14,18] lack of predictive value of response of the presence of PNH clone in our studied patients may reflect a lesser role of immune-mediated suppression of hematopoiesis. Interestingly, a second course

of IST, all with r-ATG showed a promising response rate of 53.33% among patients who were refractory to the first-line IST. The response rates of the second IST with r-ATG + CSA in different series were between 22% and 77%.[28,29] As a result, this treatment may be considered when no remission is achieved after six months of initial IST.

In conclusion, we confirmed the therapeutic benefits of IST containing ATG in patients with SAA. Amidst reports suggesting different biological activities of h-ATG and r-ATG, our retrospective study demonstrated a similar efficacy between these two products as the front-line treatment of SAA. The only predictive factor of response in our study was a lymphocyte count $\geq 1,500/\mu L$.

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