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【 ORIGINAL ARTICLE 】

Cyclosporine Therapy in Patients with Transfusion-independent Non-severe Aplastic Anemia: A Retrospective Analysis

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Abstract:

Objective The therapeutic approach for transfusion-independent non-severe aplastic anemia (NSAA) is undetermined. This study aimed to investigate the efficacy of immunosuppressive therapy (IST) for NSAA.

Methods We retrospectively reviewed 42 consecutive patients with transfusion-independent NSAA. NSAA was further divided into two stages according to the degree of cytopenia. Progression was defined as transition to a transfusion-dependent state.

Results Twelve (29%) patients received IST with cyclosporine A (CsA). Eleven (26%) patients became transfusion-dependent. In all patients, a univariate analysis revealed that a low hemoglobin level (p=0.006) and low reticulocyte count (p=0.005) were associated with a high probability of progression. The estimated transfusion-free survival (TFS) was significantly prolonged by IST among patients with advanced-stage NSAA (p=0.002), while IST did not reduce the incidence of progression in the overall cohort (p=0.349). In the non-IST group, an advanced clinical stage was significantly associated with progression (p=0.003). In contrast, the clinical stage was not related to progression in the IST group (p=0.318). None of the patients had to discontinue treatment with CsA due to renal failure.

Conclusion IST is expected to be effective in patients with advanced-stage NSAA.

Key words: aplastic anemia, immunosuppressive therapy, transfusion-independent, non-severe, natural course

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Introduction

Aplastic anemia (AA) is a clinical entity characterized by hypocellular bone marrow and pancytopenia. AA is divided into severe AA (SAA) and non-severe AA (NSAA) according to the degree of bone marrow cellularity and cytopenia. Although there has been substantial discussion about the treatment of SAA, little attention has been paid to the treatment of NSAA. Immunosuppressive therapy (IST) is not only widely used for patients with SAA (1, 2) but also contributes to a preferable outcome in patients with transfusion-dependent NSAA (3). Although the British guideline recommends non-treatment follow-up in transfusion-independent NSAA patients (4, 5), previous reports have revealed that the natural course of NSAA is poor and has a high probability of progressing to transfusion-dependent AA, especially in patients with a low absolute neutrophil count (6-8). Therefore, an appropriate interventional approach for transfusion-independent NSAA is urgently needed.

The present study aimed to clarify the efficacy of IST compared with other supportive care in patients with transfusion-independent NSAA.

Materials and Methods

We retrospectively reviewed the clinical data of patients diagnosed with transfusion-independent NSAA at The Uni-

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Table 1. Baseline Characteristics of NSAA Patients.

	Non-IST (n=30)	IST (n=12)	p value
Median age (range)	62 (27-92)	63 (24-88)	0.843
Male gender	18 (60%)	4 (33%)	0.175
Stage			
1	22 (73%)	2 (17%)	
2	8 (27%)	10 (83%)	0.001
PNH-type cells	10/20 (50%)	8/12 (67%)	0.471
Neut (mean,×10^9/L)	1.44 (0.6-2.4)	1.2 (0.5-1.9)	0.09
Hb (mean, g/L)	10.2 (7.4-13.7)	9.1 (5.8-12.7)	0.11
Reti (mean,×10^9/L)	45.1 (11-131)	47.2 (20-103)	0.78
Plt (mean,×10^9/L)	61.1 (3-150)	44 (13-210)	0.24

IST: immunosuppressive therapy, PNH: paroxysmal nocturnal hemoglobinuria, Neut: neutrophil, Hb: hemoglobin, Reti: reticulocyte, Plt: platelet

versity of Tokyo Hospital from January 2007 to October 2016. AA was defined as pancytopenia with hypocellular bone marrow meeting at least 2 of the following criteria: hemoglobin <100 g/L, platelet count <100×10^9/L, neutrophil count <1.5×10^9/L (4). SAA was defined as cytopenia with at least two of the following abnormalities: reticulocyte count <20×10^9/L, platelet count <20×10^9/L, neutrophil count <0.5×10^9/L (9, 10). Following the above-mentioned assessments, patients with AA whose cytopenia did not meet the criteria of SAA were diagnosed with NSAA. Furthermore, transfusion-independent NSAA was divided into two stages according to the Japanese classification (11). In brief, stage 2 AA had to meet at least 2 of the following criteria: reticulocyte count <60×10^9/L, platelet count <50×10^9/L, neutrophil count <1.0×10^9/L. Stage 1 AA was defined as transfusion-independent NSAA that did not meet the criteria of stage 2 AA. Transfusion-independent stage 1-2 patients were collected.

IST was defined as treatment with cyclosporine A (CsA). CsA was basically initiated at 6 mg/kg daily and then adjusted adequately according to the response or side effects of CsA with target trough concentrations between 150 and 250 μg/L. We defined progression as the development of transfusion-dependent AA. A transfusion-dependent state was defined as red blood cell and platelet transfusion in regular intervals more than once a month. Patients who did not develop transfusion-dependent AA were defined as stable patients.

The adverse events of IST were graded according to the Common Terminology Criteria for Adverse Events version 4. The existence of paroxysmal nocturnal hemoglobinuria (PNH)-type blood cells was defined as the existence of $CD55^-CD59^-$ granulocytes (>0.003%) or red blood cells (>0.005%) according to the results of a flow cytometric analysis (12, 13). The examination of PNH-type blood cells was performed at our institute and Kanazawa University Hospitals.

Statistical analyses were performed with the EZR software program, version 1.32 (modified R software) (14). Independent *t*-tests and Fisher's exact tests were used to evaluate the risk factors associated with progression to a transfusion-dependent state. The transfusion-free survival (TFS) was defined as the time from the diagnosis to the first transfusion. The TFS was estimated by the Kaplan-Meier method, and the log-rank test was used for comparisons between two groups. The level of significance was defined as two-sided ($p<0.05$). This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Review Board of our institute.

Results

Patient characteristics

We retrospectively reviewed the clinical data of 42 consecutive patients with transfusion-independent NSAA. Among them, 22 (52%) patients were men, and the median age at the diagnosis was 62 years (range: 24-92). The median follow-up period from diagnosis was 1,125 days (range: 66-3,121). The baseline characteristics of the patients are shown in Table 1. Twenty-four (57%) patients were stage 1, and the other 18 (43%) patients were stage 2. Twelve (29%) patients were treated with CsA (IST group), and the other 30 (71%) patients received watchful waiting or supportive care, including anabolic-androgenic steroids (non-IST group). A subgroup analysis of the baseline characteristics according to clinical stages is shown in Table 2. There were no significant differences between the non-IST and IST groups in either stage.

Impact of IST on the TFS

Eleven (26%) of 42 patients became transfusion-dependent. The median time to progression from the diagnosis was 49 days (range: 15-882). The estimated TFS analysis showed no significant difference between the non-IST and IST groups in the overall cohort ($p=0.349$, Figure). However, stage 2 patients were significantly more frequent in the IST group than in the non-IST group (Table 1). A TFS analysis was then performed in only the stage 2 patients because the majority of stage 2 patients received IST, and results revealed that IST significantly prolonged the TFS among patients with stage 2 disease ($p=0.002$). Although there were no significant differences, the mean trough plasma concentrations of CsA tended to be lower in patients with progression than in stable patients [246 μg/L (range: 118-370 μg/L) in stable patients and 148 μg/L (range: 117-179 μg/L) in patients with progression]. We attempted to reduce the CsA dose in patients with adequate efficacy. CsA was ultimately able to be discontinued in 5 (42%) patients without exacerbation.

Risk factors associated with progression to transfusion-dependent aplastic anemia

We then analyzed the risk factors associated with progression. In the overall cohort, univariate analyses revealed that

Table 2. Subgroup Analyses: The Baseline Characteristics of NSAA Patients.

a. Baseline characteristics of stage 1 patients.

	Non-IST (n=22)	IST (n=2)	p value
Median age (range)	58.5 (27-92)	63 (61-65)	0.672
Male gender	14 (64%)	0	0.163
PNH-type cells	8/13 (62%)	1/2 (50%)	1.000
Neut (mean, $\times 10^9$/L)	1.55 (0.9-2.4)	1.25 (1.1-1.4)	0.367
Hb (mean, g/L)	10.4 (7.4-13.7)	9.3 (6-12.6)	0.509
Reti (mean, $\times 10^9$/L)	49.7 (14.1-131)	61.7 (20-103)	0.561
Plt (mean, $\times 10^9$/L)	73.7 (13.5-150)	117.5 (25-210)	0.196

IST: immunosuppressive therapy, PNH: paroxysmal nocturnal hemoglobinuria, Neut: neutrophil, Hb: hemoglobin, Reti: reticulocyte, Plt: platelet

b. Baseline characteristics of stage 2 patients.

	Non-IST (n=8)	IST (n=10)	p value
Median age (range)	65.5 (44-90)	59 (24-88)	0.322
Male gender	4 (50%)	4 (40%)	1.000
PNH-type cells	2/7 (29%)	7/10 (70%)	0.153
Neut (mean, $\times 10^9$/L)	1.14 (0.6-1.8)	1.15 (0.5-1.9)	0.940
Hb (mean, g/L)	9.7 (7.6-12.4)	9.0 (5.8-12.7)	0.496
Reti (mean, $\times 10^9$/L)	32.4 (11-46.1)	44.3 (28.4-64.9)	0.054
Plt (mean, $\times 10^9$/L)	26.4 (3-60)	29.3 (13-47)	0.681

IST: immunosuppressive therapy, PNH: paroxysmal nocturnal hemoglobinuria, Neut: neutrophil, Hb: hemoglobin, Reti: reticulocyte count, Plt: platelet count

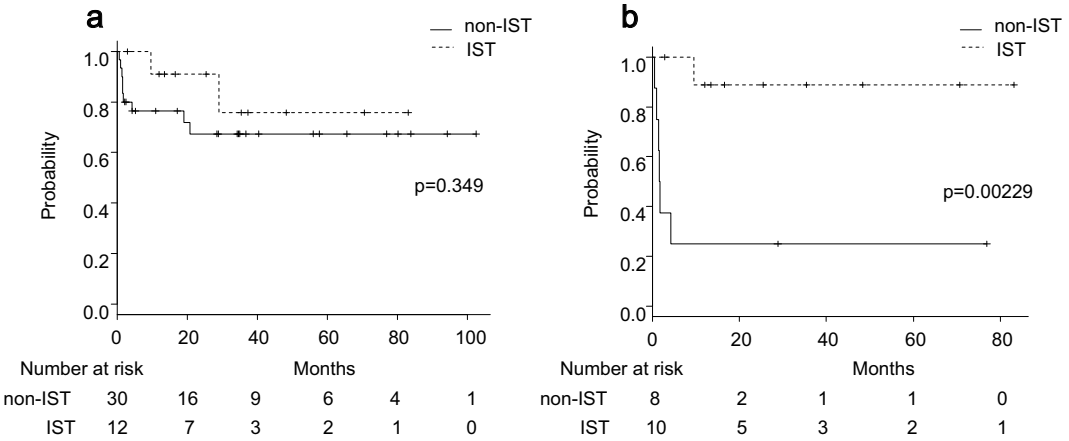

Figure. The transfusion-free survival (TFS) analyses comparing the IST and non-IST groups. a: The TFS in patients with transfusion-independent NSAA, including stage 1 and 2 NSAA. b: A TFS analysis among stage 2 NSAA.

a low hemoglobin level (p=0.006) and low reticulocyte count (p=0.005) were associated with progression (Table 3). In the non-IST group, 9 (30%) of 30 patients progressed to transfusion-dependent AA, and univariate analyses revealed that stage 2 disease (p=0.003), a low hemoglobin level (p=0.022), low reticulocyte count (p=0.020), and low platelet count (p=0.019) were associated with progression (Table 4a). In contrast, 2 (17%) of 12 patients showed progression to transfusion-dependent AA in the IST group (Table 4b). Although low hemoglobin levels were also associated with progression (p=0.027), the clinical stage was not related to progression (p=0.318) in the IST group. Interestingly, a low platelet count was associated with a favorable outcome in the IST group (p=0.021). In the current study, contrary to previous reports, the existence of PNH-type blood cells or time from diagnosis to treatment was not associated with progression in the IST group (p=1.000 and p=0.624, respectively).

Table 3. **A Comparison between the Stable and Progression Groups.**

	Stable (n=31)	Progression (n=11)	p value
Median age (range)	60 (24-92)	64 (25-90)	0.378
Male gender	16 (52%)	6 (55%)	1
PNH-type cells	13/21 (62%)	5/11 (45%)	0.465
Stage			
1	20	4	
2	11	7	0.159
Neut (mean,×10^9/L)	1.36 (0.5-2.4)	1.37 (0.6-2.4)	0.93
Hb (mean, g/L)	10.4 (7.1-13.7)	8.4 (5.8-12.4)	0.006
Reti (mean,×10^9/L)	51.4 (15.9-131.0)	29.4 (11.0-47.0)	0.005
Plt (mean,×10^9/L)	57.8 (13-150)	51.7 (3-210)	0.693
IST			
Treatment	10	2	
No treatment	21	9	0.464
Metenolone			
Treatment	3	2	
No treatment	28	9	0.593

IST: immunosuppressive therapy, PNH: paroxysmal nocturnal hemoglobinuria, Neut: neutrophil, Hb: hemoglobin, Reti: reticulocyte, Plt: platelet

Adverse events of immunosuppressive therapy

The major side effect of IST was renal dysfunction, and all 12 patients who were administered CsA showed increases in serum creatinine levels; 2 (17%) patients were grade 2, and the rest (83%) were grade 1. Although there were no significant differences, the average trough concentrations of CsA in 2 patients with grade 2 disease tended to be higher than in the other patients with grade 1 [314 μg/L (range: 258-370) and 205 μg/L (range: 117-303), respectively]. The two patients with grade 2 disease had not been administered trimethoprim-sulfamethoxazole, which is known to frequently increase the creatinine levels. Importantly, none of the patients had to discontinue CsA due to renal failure. With the exception of increased creatinine levels, there were no side effects requiring additional intervention.

Discussion

Our data indicated that IST would be effective in patients with stage 2 NSAA who were predicted to be transfusion-dependent AA in their natural course. This is the first report describing the efficacy of IST in patients with transfusion-independent NSAA.

A transfusion-dependent state provokes some medical and social problems, such as iron overload, a decline in the quality of life, and loss of social contribution. When AA progresses to transfusion-dependent state, intensive treatments, including hematopoietic stem cell transplantation, are required (5). Therefore, it is critical for patients with transfusion-independent NSAA to avoid progressing to transfusion-dependent AA. Kwon et al. previously reported that transfusion-independent NSAA was a heterogeneous entity including a group at high risk of progression and also showed that IST did not contribute to a preferable prognosis in patients with transfusion-independent NSAA in the overall cohort (8). Therefore, it is essential to identify the patients who will most benefit from IST.

Our results showed that stage 2 AA was a risk factor of progression to a transfusion-dependent state. In our analysis, the classification of the clinical stage according to the Japanese guideline contributed to the risk assessment of progression in the natural course. Furthermore, IST is the optimum therapeutic option in high-risk patients with transfusion-independent NSAA who are not receiving standard treatment. In contrast to stage 2, patients with stage 1 AA have a low risk of progression; as such, a watchful waiting strategy is reasonable in patients with stage 1 AA. However, in the present study, the existence of PNH-type blood cells or a shorter time from the diagnosis to the initiation of IST was not associated with a favorable response to IST (5, 13), probably due to the limited number of cases.

In our study, IST with CsA was generally safe and tolerable. Although increased creatinine levels were observed in all patients, there were no patients who needed to stop CsA administration. Other side effects, such as infections, leukoencephalopathy, posterior reversible encephalopathy syndrome, and hypertension, which required additional treatments, were not observed. This suggests that IST can be administered to a wide range of patients with NSAA. Furthermore, the average trough concentrations of CsA tended to be lower in patients who had progressive disease than those who did not show progression. In SAA, the trough concentrations of CsA generally range between 150 and 250 ng/mL (15). Our data suggest that maintaining trough concentrations of CsA would be also important in NSAA.

Several limitations associated with the present study war-

Table 4. Subgroup Analyses: A Comparison between the Stable and Progression Groups.

a. A comparison between the stable and progression patients with transfusion-independent NSAA receiving supportive care.

	Stable (n=21)	Progression (n=9)	p value
Median age (range)	57 (27-92)	65 (44-90)	0.093
Male gender	12 (57%)	6 (67%)	0.704
PNH-type cells	6/11 (55%)	4/9 (44%)	1.000
Stage			
1	19 (90.5%)	3 (33.3%)	
2	2 (9.5%)	6 (66.7%)	0.003
Neut (mean,$\times 10^9$/L)	1.42 (0.64-2.4)	1.48 (0.6-2.4)	0.771
Hb (mean, g/L)	10.7 (8-13.7)	9 (7.4-12.4)	0.022
Reti (mean,$\times 10^9$/L)	51.5 (15.9-131)	30.1 (11-47)	0.020
Plt (mean,$\times 10^9$/L)	71.5 (13.5-150)	36.8 (3-99)	0.019
Metenolone			
Treatment	1 (4.8%)	2 (22.2%)	
No treatment	20 (95.2%)	7 (77.8%)	0.207

IST: immunosuppressive therapy, PNH: paroxysmal nocturnal hemoglobinuria, Neut: neutrophil, Hb: hemoglobin, Reti: reticulocyte, Plt: platelet count

b. A comparison between the stable and progression patients with transfusion-independent NSAA receiving IST.

	Stable (n=10)	Progression (n=2)	p value
Median age (range)	65 (24-88)	43 (25-61)	0.282
Male gender	4 (40%)	0	0.515
PNH-type cells	7/10 (70%)	1/2 (50%)	1.000
Stage			
1	1 (10%)	1 (50%)	
2	9 (90%)	1 (50%)	0.318
Neut (mean,$\times 10^9$/L)	1.22 (0.5-1.9)	0.9 (0.7-1.1)	0.347
Hb (mean, g/L)	9.7 (7.1-12.7)	5.9 (5.8-6)	0.027
Reti (mean,$\times 10^9$/L)	51.4 (28.4-103.2)	26.3 (20.1-32.4)	0.143
Plt (mean,$\times 10^9$/L)	29 (13-47)	119 (28-210)	0.021
Metenolone			
Treatment	2 (20%)	0	
No treatment	8 (80%)	2 (100%)	1.000
Time to treatment (median, days)	53 (7-281)	42 (32-52)	0.624
Mean trough concentrations of CsA (μg/L)	246 (118-370)	148 (117-179)	0.117

IST: immunosuppressive therapy, PNH: paroxysmal nocturnal hemoglobinuria, Neut: neutrophil, Hb: hemoglobin, Reti: reticulocyte count, Plt: platelet count

rant mention. First, rapidly progressive cases that experienced progression in a relatively short duration were not excluded. As such, some of the patients who progressed to transfusion-dependent AA may have already been at the progression stage when they were initially diagnosed. Second, this study did not perform any genetic analyses. Recent reports have revealed that mutations in *PIGA* and *BCOR* and *BCORL1* correlated with a favorable response to IST and a better overall and progression-free survival (16). Further studies are warranted regarding the association between the genetic properties of NSAA and its prognosis. Third, the treatment choice was decided by each physician. There were no obvious criteria guiding the choice of treatment in this study. Finally, this study was limited by its retrospective nature and the small sample size.

In conclusion, IST with CsA in patients with transfusion-independent NSAA with a high risk of progression to transfusion-dependent AA was well tolerated and significantly prolonged the TFS. A multicenter prospective study is warranted in the future.

The authors state that they have no Conflict of Interest (COI).

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