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Immunosuppressive therapy for acquired severe aplastic anemia (SAA): A prospective comparison of four different regimens

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Objective. **This study was designed to investigate four different immunosuppressive therapy (IST) regimens as treatment of acquired severe aplastic anemia (SAA).**

Patients and Methods. **142 consecutive SAA patients were randomized to receive one of the following IST regimens: equine anti-human thymocyte immunoglobulin (E-ATG) alone (IST regimen I); E-ATG and cyclosporine A (CSA) (IST regimen II); E-ATG, CSA plus recombinant human granulocyte-macrophage colony-stimulating factor (rhuGM-CSF) and rhu erythropoietin (rhuEPO) (IST regimen III); or rabbit ATG (ATG-F), CSA, rhuGM-CSF, and rhuEPO (IST regimen IV). No repeated courses of E-ATG or ATG-F were given for nonresponders. All patients also received stanozolol or testosteron propionate.**

Results. **The overall response rate to IST regimen I was 58%. The response to IST regimen II (79%) was significantly higher ($p = 0.04$), more rapid and complete than after IST regimen I. The response rate to IST regimen IV (53%) was significantly lower than that of IST regimen III (73%, $p = 0.039$). The additional use of growth factors did not reduce early deaths and did not accelerate hematopoietic recovery after IST. Of the 142 patients enrolled in this trial, 92 (65%) are alive at a median follow-up time of 102 months (range, 54–166 months). The 5-year actuarial survival for IST regimens I, II, III, and IV was 58%, 81%, 80%, and 66%, respectively.**

Conclusion. **The combination of E-ATG and CSA remains the best combination for the treatment of SAA patients, producing a survival advantage at 5 years. The addition of growth factors did not improve these results. Rabbit ATG-F appeared less effective than E-ATG.**

Acquired severe aplastic anemia (SAA) is a rare disease characterized by pancytopenia and a hypoplastic bone marrow [1–3]. Allogenic bone marrow transplantation (allo-BMT) can cure the majority of transplanted patients [4–6], but most patients are ineligible for this procedure because they have no histocompatible sibling donor, or because of age restriction. The alternative treatment is immunosuppressive therapy (IST), generally anti-lymphocyte globulin (ALG) or anti-thymocyte globulin (ATG) or cyclosporine A (CSA), which produces hematologic response rates of 40 to 50% [7–13]. Modified IST protocols have combined ALG or ATG with CSA to achieve response rates of 60 to 80% in patients with SAA [14–16]. Despite this progress, many patients may become refractory to IST or develop late clonal diseases, such as paroxysmal nocturnal hemoglobinuria (PNH) or myelodysplastic syndrome (MDS). Thus, the treatment of SAA still presents a therapeutic challenge even to experienced clinicians.

Another recent avenue of investigation involves the use of hematopoietic growth factors (HGFs). Recombinant human granulocyte-macrophage colony-stimulating factor (rhuGM-CSF) was first used to treat patients with SAA in the late 1980s [17]. Subsequent clinical trials showed that rhuGM-CSF or recombinant human granulocyte colony-stimulating factor (rhuG-CSF) administered for a more prolonged period to patients receiving IST may produce very encouraging results, namely enhancement of hematological

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recovery and reduced early mortality [18–20]. There is limited experience with the use of recombinant human erythropoietin (rhuEPO) in SAA [21]. An earlier report described clinical responses to rhuEPO and rhuGM-CSF in patients with moderate or refractory AA [22]. Moreover, further insights into the cellular actions of cytokines suggested that rhuEPO act synergistically with rhuGM-CSF [23], which might enhance erythropoiesis and bone marrow restoration in patients with SAA [24].

We therefore undertook this prospective trial to compare the hematologic responses of several IST regimens, and especially the potential benefit of administrating rhuHGF combination, namely rhuGM-CSF plus rhuEPO, in *de novo* patients with SAA.

Patients and methods

Patients

One hundred and forty-two hospitalized patients referred to our institute with the diagnosis of SAA between August 1991 and October 2000 were enrolled in this study to be evaluated. All patients or their parents or legal guardians were informed and provided signed informed consent. This study was approved by the Institutional Committee for Medical Care and Safety. Patients with SAA were as defined by the criteria of Camitta et al. [25] and included bone marrow hypoplasia (cellularity less than 25%) and at least two of the following three laboratory abnormalities: a corrected reticulocyte count below 1%, an absolute neutrophil count (ANC) less than 0.5×10^9/L, and a platelet count less than 20×10^9/L. Patients were classified as very SAA (VSAA) if they met the criteria for SAA and had an ANC less than 0.2×10^9/L. In children under the age of 18 years, a cytogenetic diagnosis test to eliminate Fanconi's anemia was performed. Patients with the existence of PNH clone were excluded from this trial. This series of SAA patients were ineligible for related or unrelated bone marrow transplantation (BMT) because of lacking fully HLA-matched siblings, or inability to locate fully HLA-matched unrelated donors, or age (>50 years), or other personal reasons. The pretreatment clinical characteristics of the enrolled 142 patients are outlined in Table 1. The 142 patients ranged in age from 2 to 71 years, median disease duration at the time of IST was 58 days, and 45 patients met criteria for VSAA. The etiology of the 142 patients was as follows: idiopathic, 92 (64.8%); hepatitis-related, 27 (19.0%); drug- or chemical-related, 19 (13.4%); and others, 4 (2.8%). There were no differences in baseline characteristics among the above-mentioned groups.

IST schedule

This was an open-label study. All patients randomly received one of the four different IST regimens, together with androgen as an adjunct.

IST regimen I. Equine anti-human thymocyte immunoglobulin (E-ATG, Lymphoglobuline), provided by Institute Pasteur Merieux C (Lyon, France), was administered by slow intravenous infusion at a dose of 12 mg/kg/day for 5 consecutive days, together with prednisone (pred), 1 mg/kg/day orally on days 1 through 15, and then tapering the dose and stopping on day 30.

IST regimen II. E-ATG was combined with cyclosporine A (CSA, Sandoz Pharmaceuticals Corp., Basel, Switzerland, or Novartis Pharma GmbH, R.P. Scherer GmbH & Co. KG, Eberbach, Germany after 1996); the use of E-ATG was as mentioned above; oral CSA, 5 mg/kg/day daily, was added to E-ATG for 6 months, and maintained at a dose of 2.5 mg/kg/day daily for additional 6 months. The patients' plasma levels of CSA were not measured.

IST regimen III. E-ATG, CSA, plus rhuGM-CSF and rhuEPO; the uses of E-ATG and CSA were as mentioned above; rhuGM-CSF and rhuEPO briefly as follows: treatment with both cytokines was initiated on day 31; rhuGM-CSF (Schering-Plough, Cork, Ireland) 5 μg/kg/day subcutaneously was administered 3 days a week for the first month, 2 days a week for the second month, and 1 day a week during the third month; rhuEPO (Amgen-Roche, Thousand Oaks, CA, USA, or Kirin, Tokyo, Japan after 1998), 100 units/kg/day, was administered by intravenous infusion 3 days a week for the first month, 2 days a week for the second month, and 1 day a week during the third month.

IST regimen IV. A different immunosuppressive agent, namely rabbit anti-human T lymphocyte immunoglobulin (ATG-F), provided by Frensenius HemoCare Immune Therapy GmbH (Bad Homburg, Germany), 5 mg/kg/day, was administered by slow intravenous infusion on days 1 through 5; the uses of CSA, rhuGM-CSF, and rhuEPO were as mentioned above.

Basic therapies

All 142 patients received oral Stanozolol, 0.15 mg/kg/day daily, or testosteron propionate (Triolandren, Ciba-Geigy, Basle, Switzerland), 4 mg/kg/day intramuscularly (IM) 2 days a week, vitamin B_{12} 10 μg/kg/day, IM 1 day a week, and oral folic acid 0.5 mg/kg/day daily. All of these medicines were maintained for more than 1 year. None of the patients showed lower serum level of vitamin B_{12} or folic acid pre-enrollment.

Supportive care

All patients were cared for in rooms with laminar air flow (LAF) 1 week before IST and continued until an appropriate neutrophil response as ANC $\geq 1.0 \times 10^9$/L. No patients received prophylactic antibiotic support. Red blood cells (RBC) were transfused when the hemoglobulin level was less than 80 g/L, and platelets were transfused when the blood platelet count was less than 10×10^9/L, or less than 20×10^9/L in the presence of bleeding and/or fever.

Follow-up research

All of the patients were evaluated at baseline and strictly followed up for every 3 to 6 months after IST by rehospitalizing at our institutional hospital. At each evaluation, clinical assessments were performed, and a complete blood count (CBC), platelet count, and bone marrow aspiration and biopsy were obtained, also with the colony assay of bone marrow hematopoietic progenitor cells, including burst-forming unit-erythroid (BFU-E) and colony-forming unit–granulocyte/macrophage (CFU-GM) by the method described previously [26]. Clinical assessments included determinations of red blood cell and platelet transfusion requirements, the

Table 1. Characteristics of all 142 patients with SAA

	All SAA (n = 142)	Group I (n = 33)	Group II (n = 47)	Group III (n = 30)	Group IV (n = 32)
Gender, male (%)	103 (72.5%)	24 (72.7%)	36 (76.6%)	22 (73.3%)	21 (65.6%)
Age (yr), median (range)	34 (2–71)	36 (6–63)	35 (8–71)	36 (5–68)	29 (2–66)
Duration before treatment (median days)	58	48	62	51	39
Severe infections (no/yes)	120/22	27/6	42/5	25/5	26/6
Severe hemorrhages (no/yes)	131/11	30/3	44/3	28/2	29/3
Peripheral blood cell count before treatment (median)					
RC ($\times 10^9$/L)	8.1	8.2	9.0	6.9	7.2
ANC ($\times 10^9$/L)	0.40	0.41	0.39	0.43	0.40
PLT ($\times 10^9$/L)	12	11	14	11	15
ANC $< 0.2 \times 10^9$/L	45 (31.7%)	12 (36.4%)	14 (29.8%)	10 (33.3%)	9 (28.1%)

time to transfusion independence, infectious complications and infection-related deaths, and the overall response rates. In addition, cytogenetics analysis and glycosyl-phosphatidylinositol-anchored protein (GPI-APs) detection using flow cytometry (anti-CD55, anti-CD59) were repeatedly performed in surviving patients to monitor the development of late clonal complications, including PNH and MDS after IST.

Responses

Patients were classified as follows: complete responders: transfusion independence, with a hemoglobulin level of at least 110 g/L, a neutrophil count greater than 1.5×10^9/L, and a platelet count greater than 100×10^9/L; partial responders: transfusion independence with a hemoglobulin level of at least 80 g/L, a neutrophil count greater than 0.5×10^9/L, and a platelet count greater than 20×10^9/L. Persistence of transfusion requirement or death was evidence of no response.

Statistical analysis

The nonparametric Wilcoxon's rank sum test was used to assess differences among treatment groups in recoveries of peripheral blood cell counts, bone marrow numbers of colony-forming units, and intervals between treatment and responses; while χ^2 tests were used to compare dichotomous variables such as infection rates and response rates. Actuarial survival was calculated using the method of Kaplan-Meier, and comparisons made using the log-rank test. A p value less than 0.05 was considered statistically significant.

Results

Response rates

All 142 patients received only one course of E-ATG or ATG-F; no repeated courses of E-ATG or ATG-F were given for the nonresponders 6 months after IST due to financial problems, which could allow us to straightforwardly compare the outcomes produced by each IST regimen on SAA patients. The response rates of the total 142 patients up to the endpoint of follow-up were as follows: 69 (49%) patients achieved complete remission (CR), 26 (18%) patients partial remission (PR), the remaining 47 (33%) no response (NR); the overall response rate was 67%. The comparison of response rates among the four groups was as seen in Table 2. The overall response rates and CR rates between Group II and Group III were comparative; both the overall response rates and CR rates in Group II (p values were 0.038 and 0.040 vs corresponding Group I data, respectively) and Group III (p values were 0.040 and 0.032 vs corresponding Group I data, respectively) were significantly higher than those in Group I; but somewhat unexpectedly, the overall response rate (53%) and CR rate (31%) in Group IV, which were very similar to those in Group I, were significantly lower than those in Group III (p values were 0.039 and 0.035, respectively).

Table 2. Comparison of response rates among four groups

Groups	Cases	IST regimen	Response rates				p value*
			CR (%)	PR (%)	NR (%)	The overall response rate (%)	
Group I	33	E-ATG	13 (39.4)	6 (18.2)	14 (42.4)	57.6	
Group II	47	E-ATG+CSA	28 (59.6)	9 (19.1)	10 (21.3)	78.7	0.038
Group III	30	E-ATG+CSA+rhuGM-CSF+rhuEPO	18 (60.0)	4 (13.3)	8 (26.7)	73.3	0.040
Group IV	32	ATG-Fresenius S+CSA+rhuGM-CSF+rhuEPO	10 (31.3)	7 (21.9)	15 (46.8)	53.2	>0.05

*Compared with that of Group I.

Table 3. Comparisons of recovery of hematopoiesis among four groups

	Group I	Group II	Group III	Group IV
Recovery of peripheral blood cell count (median days)*				
RBC transfusion independence	167 (20–420)	105 (20–370)#	117 (46–300)#	137 (82–280)
PLT transfusion independence	134 (28–370)	116 (22–460)	112 (34–340)	119 (60–280)
ANC $> 1.0 \times 10^9$/L	89 (20–310)	94 (21–362)	87 (38–240)	135 (30–240)#
Interval between treatment and response (median days)*	199 (90–360)	146 (21–420)#	156 (58–351)	192 (54–390)
Numbers of BM CFC (mean ± s)*				
BFU-E	13.6 ± 7.0	23.0 ± 11.6#	24.0 ± 17.7#	13.3 ± 6.1
CFU-GM	1.6 ± 5.0	21.3 ± 9.0#	17.2 ± 9.4	11.4 ± 6.7

*Compared with the corresponding Group I data.
#indicates $p < 0.05$.

Early infection-related mortality

Early mortality (within 100 days after IST onset), a major problem associated with IST for SAA, is mainly caused by invasive infection. All patients were closely monitored for the occurrence of infections, confirmed by clinical signs or symptoms and/or laboratory evaluation. In this series of 142 patients, 24 (17%) died within 6 months. Of them, 14 and 7 patients died of bacterial and fungal sepsis, respectively. The numbers of early infection-related mortality in Group I, Group II, Group III, and Group IV were 7 (21%), 3 (6%), 4 (13%), and 7 (22%), which suggested that additional uses of rhuHGFs did not reduce the rate of early infection-related mortality.

Recovery of hematopoiesis

The comparisons of recovery of hematopoiesis among the four groups were as seen in Table 3. The levels of bone marrow BFU-E and CFU-GM shown in Table 3 referred to the CFC numbers in 2×10^5 bone marrow mononuclear cell culture 2 years after IST. With regard to speed and degree of the recovery of hematopoiesis, median times of interval between treatment and response for the responding patients in Group II and Group III were more rapid, and also with more complete improvement of bone marrow BFU-E and CFU-GM numbers than the counterparts in Group I and Group IV.

Survival

Of the 142 patients enrolled into this trial, 92 (65%) are alive at a median follow-up time of 102 months (range, 54–166 months). The actuarial 12-month survivals in Group I, Group II, Group III, and Group IV were 70%, 91% ($p = 0.01$ vs the corresponding Group I data, the same as follows), 83% ($p = 0.2$), and 78% ($p = 0.4$), respectively. A long-term survival advantage in favor of the combination of E-ATG (but not for ATG-F) and CSA with or without rhuGM-CSF plus rhuEPO over E-ATG alone was observed at 60 months. The 60-month survival rates for those groups were 58%, 81% ($p < 0.001$), 80% ($p = 0.002$), and 66% ($p = 0.505$), respectively, estimated by the method of Kaplan-Meier (Fig. 1).

Multivariate analysis on response

Responses were equally distributed when patients were stratified for age, sex, etiology, peripheral blood reticulocyte and platelet levels before treatment, the severities of infections and hemorrhages, and interval between diagnosis and treatment. The ANC at the time of diagnosis was the only significant prognostic variable for response: the response rates of patients with ANC less than 0.2×10^9/L in Group I, Group II, Group III, and Group IV were 25.0% (3/12), 71.4% (10/14, $p = 0.031$, compared with that of Group I, same as follows), 60.0% (6/10, $p = 0.045$), and 33.3% (3/9, $p > 0.05$), respectively.

Adverse events and toxicity

Generally, the addition of rhuGM-CSF plus rhuEPO to the E-ATG/ATG-F and CSA treatment regimens was well tolerated. Repeated renal function tests showed that both levels of blood urea nitrogen and creatinine in patients receiving the prolonged course of CSA were normal. Fifteen

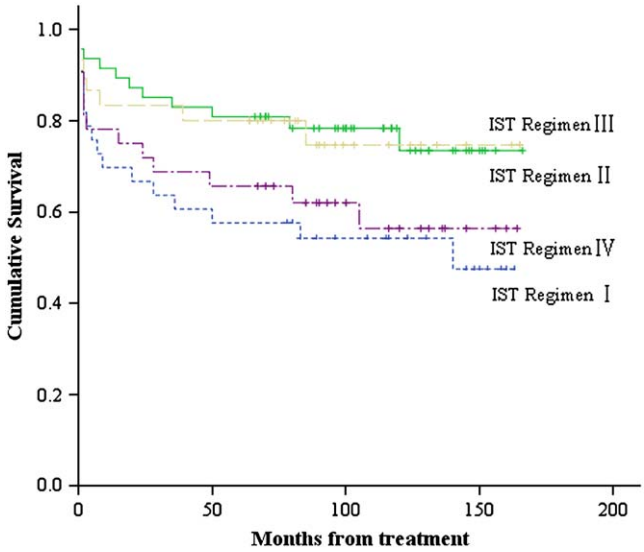

Figure 1. Comparing results of Kaplan-Meier probability of survival for patients with SAA receiving four IST regimens. Survival was calculated as of the data of last contact.

(24.2%), 1 (1.6%), and 9 (14.5%) of the 62 patients receiving rhuHGFs experienced low-grade fevers, Sweet syndrome, and hypertension, respectively, during rhuGM-CSF plus rhuEPO administration. The fevers resolved within 4 hours without specific treatment. The patient with Sweet syndrome recovered within 2 weeks after oral pred. rhuEPO-related hypertension was well controlled by appropriate intervention. In addition, androgen-related, and also transient elevated glutamic-pyruvic transaminase levels at a median of 2.3 times (range, 1–4.3 times) greater than the normal range were observed in 55 out of the 142 patients (38.7%); other androgen-related side effects included weight gain and increased bilirubin and alkaline phosphatase serum levels. Proper adjustment of androgen dose could overcome its side effects without other intervention.

Late clonal complications

The small PNH clone granulocyte cells (the percentages of $CD59^-$ cells ranged from 3.27 to 7.59%) were detected by flow cytometry in 4 (1 in Group II and another 3 in Group III) of the responding patients without overt hemolysis. Until the last time point of follow-up, no patients had developed late clonal complications.

Discussion

For most of its history, SAA has had a dire reputation. In the 1970s, the observation of autologous marrow recovery after treatment with anti-lymphocyte sera (ALS) suggested that nonreplacement therapy could restore marrow function. This strongly suggests that most SAA patients have immune-mediated marrow failure, mediated by cytotoxic T lymphocytes that are detectable in blood and marrow [27]. These cells produce negative regulators, such as cytokines γ-interferon and tumor necrosis factor-α, both of which inhibit progenitor cell growth and the generation of long-term culture-initiating cells, an in vitro surrogate for hematopoietic stem cell [28]. Increasingly refined IST regimens and intensified IST (namely ALG/ATG combined with CSA), as well as improved transfusion support and modern antimicrobials, have produced spectacular hematologic improvement and long-term survival rate.

In this clinical setting, no nonresponders proceeded to any forms of salvage therapies, such as repeated courses of IST, high-dose cyclophosphamide, or BMT, which offered us the unique opportunity to evaluate the clinical efficacy of the four IST regimens as first-line therapy on SAA patients; meanwhile, our study could exclude any discrepancies of outcomes produced by different IST regimens hidden in cross-over clinical trials.

One important question is whether growth factors are essential to achieve the desirable outcome for patients with SAA after IST. Neither deficient stromal cell function nor growth factor production is central to the etiology of SAA [29,30]. Despite the lack of objective support for their use, and possible delay in commencing definitive therapy with the increased risks of alloimmunization and infections, a series of growth factors, such as rhuG-CSF, rhuGM-CSF, and rhuEPO are now frequently offered to patients with SAA. Some of these can shorten the period of neutropenia post–marrow transplantation, and they may be beneficial when used as an adjunct in an immunosuppressive regimen to relieve neutropenia as a major cause of infections and toxicity [18–20]. In the present clinical study the combination of rhuGM-CSF plus rhuEPO did not reduce the rate of early infection-related mortality, did not accelerate the recovery of hematopoiesis, and did not increase response rate after IST, nor proved beneficial on survival. Although the number of the enrolled patients is small, and unfortunately this study was discontinued by the end of 2000 because of unavailability of rhuGM-CSF in Mainland China, still our study suggests that rhuGM-CSF plus rhuEPO is not essential and probably should not be included in IST regimen for SAA. Our results were in agreement with an earlier prospective trial testing the effect of ALG with or without rhuGM-CSF on patients with SAA; patients were most pretreated in that study, and survival or responses were not improved in the rhuGM-CSF arm [31]. Another such trial has been conducted and has shown earlier neutrophil recovery and fewer infections, but has failed to show a higher rate of trilineage response or survival in the rhuG-CSF-treated group [32]. So far, no formal proof of a significant advantage with SAA receiving rhuHGFs in association with intensified IST is available.

Another important aspect that deserves mentioning was the response rate produced by IST regimen IV; this unsatisfactory result might be due to the undesirable in vivo activity of ATG-F on bone marrow failure. Currently therapeutic ALS preparations for SAA are purified from animal plasma after immunization with children's thoracic duct lymphocytes, e.g., E-ALG (Lymphoser, from the Swiss Serum and Vaccine Institute, Berne, Switzerland) and thymocytes, e.g., E-ATG (from Institut Pasteur Merieux, Lyon, France; and from Upjohn, Kalamazoo, MI, USA), both of which appeared to be capable of producing much higher efficacy as treatment for SAA [3,13–16], while ATG-F is isolated from the serum of rabbits immunized with human T lymphoblasts from the human Jurkat cell line instead of children's thoracic duct lymphocytes or thymocytes. The mechanisms of ALS preparations' actions are complex and not fully understood. It was believed that the mechanisms of ALG/ATG are involved in their multi-effects on hematopoiesis, including immunosuppressive effect [33], immunostimulatory effect [34], and direct effect on hematopoietic stem/progenitor cells [35,36], instead of immunosuppressive effect alone for ATG-F [37]. The differences of activity among those ALS preparations might deserve (or require) further study.

Secondary clonal diseases are relatively common after IST for SAA patients [38,39]. In this clinical study, only

4 responding patients after IST demonstrated measurable levels of GPI-AP-negative cells; no one patient has developed into late clonal disease, which might be due to our strict measurements for diagnosis of SAA.

In summary, intensified IST (namely ALG/ATG combined with CSA) remains the treatment of choice for acquired SAA.

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