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Treatment of severe aplastic anemia with antilymphocyte globulin, cyclosporine and two different granulocyte colony-stimulating factor regimens: a GITMO prospective randomized study

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A B S T R A C T

Background and Objectives. In a previous study we showed that patients with severe aplastic anemia (SAA) treated with anti-lymphocyte globulin (ALG), cyclosporin (CyA) and granulocyte colony-stimulating factor (G-CSF) 5 μg/kg/day had an encouraging outcome. However, failure to respond, delayed responses, partial responses, relapses and early deaths remain signifcant problems. The aim of the present study was to test whether a higher dose of G-CSF (10 μg/kg/day) would reduce these complications.

Design and Methods. This was a multicenter prospective trial in 77 SAA patients treated with horse ALG (15 mg/kg/day day1-5) and CyA (5 mg/kg/day day 1-180). Patients were randomized to receive G-CSF 5 μg/kg/day (n=38, group A) or 10 μg/kg/day (n=39, group B) from day +1 to day +30. All patients then received G-CSF 5 μg/kg/day from day +31 to day +90. The primary end point of this study was response at day +120. Secondary end points were early deaths, blood counts at day +120, and survival.

Results. At day +120 responses were classified as absent, partial, and complete in 12, 22, and 4 patients in group A and in 23, 7, and 9 patients in group B (p=0.001). At last follow-up these figures were respectively 9, 12, and 17 vs 19, 2, and 18 (p=0.004). Thirteen patients (5 in group A and 8 in group B) died before day 120 (p=0.3). Median peripheral blood counts on day 120 were comparable in the two groups: Hb 10.5 and 9.5 g/dL in group A and B, respectively (p=0.6), Neutrophil counts were 2.4 vs 1.9×10^9/L in groups A and B (p=0.4) and platelet counts were, respectively, 42 vs 36×10^9/L (p=0.3). The actuarial survival at 4 years is 72% in group A and 67% in group B (p=0.3).

Interpretation and Conclusions. Increasing the dose of G-CSF does not appear to reduce early deaths, does not improve peripheral blood counts nor survival, and may reduce the response rate in patients with SAA receiving ALG and CyA.

Key words: acquired aplastic anemia, immunosuppression, G-CSF dose.

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Acquired severe aplastic anemia (SAA) is a rare disease, defined as peripheral blood pancytopenia associated with hypocellularity of the bone marrow.[1] In most cases, bone marrow failure is thought to result from an immune-mediated mechanism which leads to T-cell activation and release of inhibitory cytokines with subsequent destruction of hematopoietic progenitor cells.[2] Immunosuppression is the treatment of choice in older patients with a relatively high neutrophil count,[3] as well as in patients who would be eligible for transplantation by age and blood cell counts, but lack a suitable donor. Antilymphocyte globulin (ALG) is the single most effective drug in SAA, as shown by prospective randomized trials.[4-6] Two prospective trials have tested ALG alone versus combination treatment with CyA[7] or androgens:[8] combined therapy was superior to ALG alone in terms of response but not in terms of survival, as shown also by a recent update of the German randomized trial.[7] Because response and transfusion independence are important outcomes of SAA treatment, the combination of ALG+CyA is currently the treatment of choice.[9]

Recombinant hematopoietic growth factors, such as granulocyte colony-stimulating factor (G-CSF), are not indicated as single therapy in patients with marrow failure.[10] G-CSF has been tested in combination with ALG+CyA in one pilot study at the dose of 5 μg/kg/day for 3 months:[11] response and survival were encouraging. However, a prospective randomized trial failed to show a significant survival benefit for patients receiving ALG+CyA+G-CSF compared to ALG+CyA alone:[12] that study showed better neutrophil response in G-CSF recipients and improved failure-free

survival. There was no major difference in infections and infection-related deaths between the two groups. Finally the long-term effects of G-CSF treatment are uncertain and conflicting results have been reported on the risk of late clonal disorders.[12-14]

The hypothetical mechanism by which G-CSF may be helpful is through mobilization of hematopoietic progenitor cells: indeed a relatively large number of colony-forming cells can be collected from the peripheral blood of SAA patients after prolonged exposure to daily G-CSF and these cells can then be cryopreserved.[15] One patient has been reinfused with cryopreserved autologous peripheral blood cells after conditioning with low dose fludarabine and cyclophosphamide and is currently transfusion-independent 4 years after the graft (*unpublished data*). Higher doses of G-CSF mobilize larger numbers of hematopoietic progenitor cells (HPC) in normal donors, as shown by comparing CD34 counts in subjects receiving 5 μg or 10 μg/kg/day of G-CSF.[16]

The present study was designed to test whether G-CSF at the dose of 10 μg/kg/day is better than G-CSF 5 μg/kg/day in SAA patients receiving conventional immunosuppression with ALG, CyA and steroids. The primary end-point was response at 120 days. Secondary end-points were: peripheral blood counts and mortality at day +120, and actuarial survival in a series of newly diagnosed patients with acquired SAA.

Design and Methods

Design of the study

The study was designed as a prospective, multicenter, randomized trial in patients with acquired SAA treated with immunosuppression, comparing two schedules of G-CSF: 5 μg/kg/day s.c., days 1-90 or 10 μg/kg/day s.c. days 1-30, followed by 5 μg/kg/day s.c., days 31-90. Only newly diagnosed patients were included. The inclusion criteria were severe aplastic anemia (neutrophil counts $\leq 0.5\times10^9$/L) and an age from 1 to 80 years. The diagnosis had to be confirmed by bone marrow biopsy and cytogenetic analysis performed before treatment. Additional criteria were the absence of a preceeding malignancy, absence of major organ impairment, and no chromosomal abnormality or chromosomal breakage. Informed written consent was obtained from all patients or their parents, and the study was approved by the ethics committee of each participating institution.

End-points of the study

The primary end-point of the study was response rate at 120 days in the two arms. Secondary end-points were peripheral blood counts on day 120, number of death within 120 days and actuarial survival.

Table 1. Clinical characteristics of 77 SAA patients randomized to receive IS with different schedules of G-CSF.

Treatment arm	*A* *5 μg G-CSF*	*B* *10 μg G-CSF*	*p*
N.	38	39	
Age at diagnosis			
median (years)	20	21	0.46
range (years)	2-78	2-74	
Gender			
male/female	27/11	24/15	0.37
Patients with neutrophils			
0-0.20×10⁹/L	17	18	
≥ 0.21×10⁹/L	21	21	
Platelet counts			
median (×10⁹/L)	6	9	0.55
range (×10⁹/L)	(1-49)	(1-33)	
Infection: yes/no	12/26	11/28	0.74
Hemorrhage:yes/no	15/23	16/23	0.89
Interval diagnosis-treatment			
median (days)	12.5	15	0.8
range (days)	(0-64)	(0-1095)	

Treatment

The two treatment regimens consisted of horse antilymphocyte globulin. (IMTIX SangStat) 15 mg/kg/day on days 1, 2, 3, 4 and 5; cyclosporin 5 mg/kg/day from day 1 for at least 180 days and then tapered slowly, methylprednisolone (6 Mpred) 2 mg/kg/day for 5 days, and then the dose halved every 5 days until discontinuation on day +30; granulocyte colony stimulating factor 5 μg/kg/day s.c., days 1-90 (arm A) or 10 μg/kg/day s.c. days 1-30, followed by 5 μg/kg/day s.c., days 31-90 (arm B).

Thirty days of high-dose G-CSF was chosen as this is the minimum time for hematopoietic progenitor mobilization in SAA patients.[15] Supportive care included oral ciprofloxacin during the ALG treatment, oral fluconazole and transfusion support as required.

Patients

Seventy-seven patients with acquired aplastic anemia entered the study. Their median age at diagnosis was 21 years (range 2-78 years). Fifty-one were male and 26 female. Aplastic anemia was very severe in the majority of them (median neutrophil count at diagnosis = 0.2×10^9/L). Twenty-three patients had infections and 31 had hemorrhages. The median interval between diagnosis and immunosuppressive treatment was 13 days (range: 0 to 1095 days). Thirty-eight were randomized in arm A (G-CSF 5 μg/kg/day) and 39 into

Arm B (G-CSF 10 μg/kg/day). The clinical characteristics of the two groups were comparable (Table 1).

Responses

Complete responses were defined as transfusion independence associated with a hemoglobin (Hb) concentration greater than 11 g/dL, neutrophil counts greater than 1.5×10^9/L and a platelet count greater than 100×10^9/L. We defined partial response as transfusion independence associated with Hb greater than 8 g/dL, neutrophil counts greater than 0.5×10^9/L and a platelet count greater than 30×10^9/L. Transfusion dependence was taken as evidence of no response.[11]

Relapse

A patient was considered in relapse if he or she required transfusions of red blood cells or platelets after having been independent from transfusions for at least 3 months. Some patients showed declining peripheral blood counts that could be controlled by increasing the dose of CyA and did not require transfusions; these episodes were not recorded as relapse.

Failure-free survival

Failure was defined as one of the following events: receiving a second course of ALG, relapse after response, development of clonal disease (acute myeloid leukemia, myelodysplasia), bone marrow transplantation (BMT) or death.

Statistical analysis

The Student's T and Mann-Whitney tests were used for continuous variables, χ^2 2×2 tables and the log-rank test for time-dependent variables and Kaplan Meier curves were used for actuarial survival. The number cruncher software (NCSS, version 5.0, JL Hintze, Kaysville, UT, USA) was used to perform analyses. Survival curves were drawn censoring patients at the time of transplant, when performed. Because patients may experience more than one failure event, such as relapse followed by retreatment with ALG or BMT, failure-free survival was computed using the first event occurring in time.

Results

Response

The outcome with respect to the primary-end point is shown in Table 2. At the analysis 120 days after the start of treatment, complete trilineage hematologic recovery was seen in 13 patients (17%) with no significant difference between the two arms (4 and 9 in arm A and B, respectively). Twenty-nine patients (38%) had a partial respondse: 22 in arm A and 7 in arm B.

Table 2. Primary and secondary end-points of the study.

Treatment arm	*A 5 μg G-CSF*	*B 10 μg G-CSF*	*p*
Number of patients	38	39	
Primary end point			
Response at 120 days			
No response	12	23	
Partial response (PR)	22	7	
Complete response (CR)	4	9	0.003
PR +CR%	68%	41%	0.01
Secondary end-points			
N. deaths within 120 days	5	8	0.28
Peripheral blood counts on day +120			
Hb (g/L)	10.5 (6-12.6)*	9.5 (5.8- 14.4)°	0.6
PMN ($\times10^9$/L)	2.4 (0.5-10)	1.9 (0.1-8.9)	0.4
Plt ($\times10^9$/L)	42 (5- 142)	36 (4-180)	0.3
Responses at last follow up (FU)			
Median FU months (range)	49 (1-96)		
No response	9	19	
Partial response	12	2	
Complete response	17	18	p=0.004
Causes of failure			
Relapse	4		3
Second course of ALG	7		7
Acute myeloid leukemia	0		2
Clonal cytogenetic abnormality	2		0
Bone marrow transplant	4		6
Total number of deaths	9		13

*ns: $p \geq 0.5$; *22 patients; °26 patients.*

Thirty-five patients (45%) showed no recovery (n=22) or died early (n=13) (Table 2). The distribution of responses (no/partial/complete) was significantly different in the two treatment arms in favor of the 5 μg/kg dose of G-CSF (p=0.003).

At the last follow-up, at a median of 49 months (range 1-96) after the start of treatment, we re-evaluated response status in the patients in the two treatment arms (Table 2): there had been no response in 10 and 19 of the patients in the 5 μg/kg and 10 μg/kg arms, partial responses in 13 and 2 and complete responses in 15 and 18 (p=0.004) always in favor of 5 μg/kg of G-CSF.

Peripheral blood counts on day +120

One of the secondary end-points was blood counts on day +120, which were available for 22 patients in

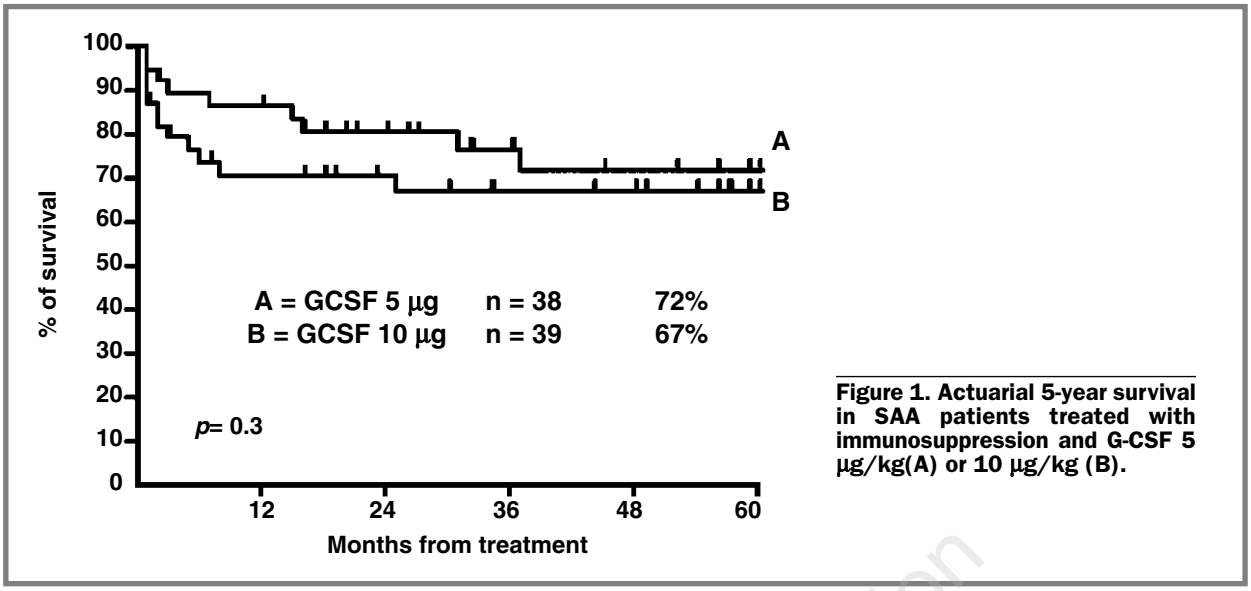

Figure 1. Actuarial 5-year survival in SAA patients treated with immunosuppression and G-CSF 5 μg/kg(A) or 10 μg/kg (B).

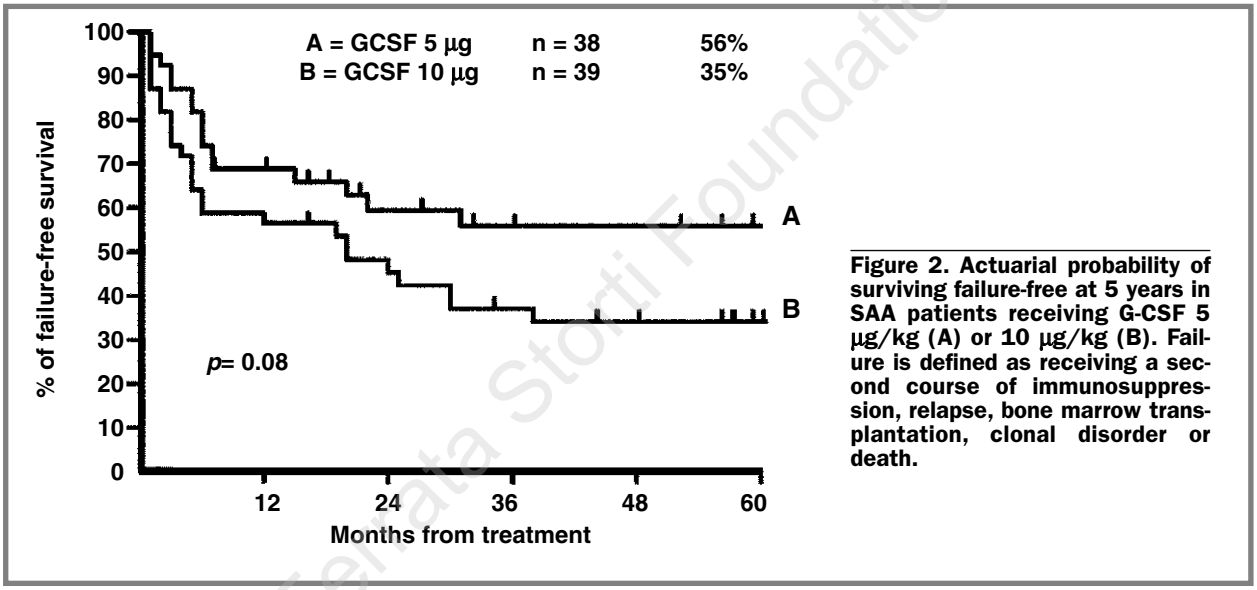

Figure 2. Actuarial probability of surviving failure-free at 5 years in SAA patients receiving G-CSF 5 μg/kg (A) or 10 μg/kg (B). Failure is defined as receiving a second course of immunosuppression, relapse, bone marrow transplantation, clonal disorder or death.

group A and 26 patients in group B. These are summarized in Table 2 and show no major differences in Hb, neutrophil and median platelet levels between the two arms.

Causes of death

Twenty-two patients (9 in arm A and 13 in arm B) died after a median of 85 days (range 10-2278 days) of treatment. In arm A, 6 patients died of infections, 1 of cytopenia, 1 of hemophagocytic histiocytosis and 1 of hemorrhage. In arm B, 7 died of infection, 4 of hemorrhage, 1 of acute myeloid leukemia and 1 of an unknown cause.

Survival

The actuarial survival at 4 years is 72% in arm A and 67% in arm B (p = 0.3) (Figure 1). The survival rate among patients with very severe aplasia (neutrophils less than 0.2×10^9/L) was 82% and 70% in arm A and arm B, respectively (p=0.3). The corresponding rate for patients with a neutrophil count between 0.2 and 0.5×10^9/L was 66% in both groups A and B (p=0.5).

Failure-free survival

There were 26 failures in group A and 31 failures in group B, failure being identified, as described previously, as second treatment, transplantation, relapse, acute leukemia or clonal evolution (Table 2). Because an individual could have experienced more than one failure, for example relapse, followed by second ALG followed by transplantation, failure free-survival was computed taking into account the first failure in time. When this was done there were 16 and 25 failures in arms A and B, respectively (p=0.05). After 4 years, the failure-free survival rate was 56% and 35% in arms A and B, respectively (p=0.08) (Figure 2).

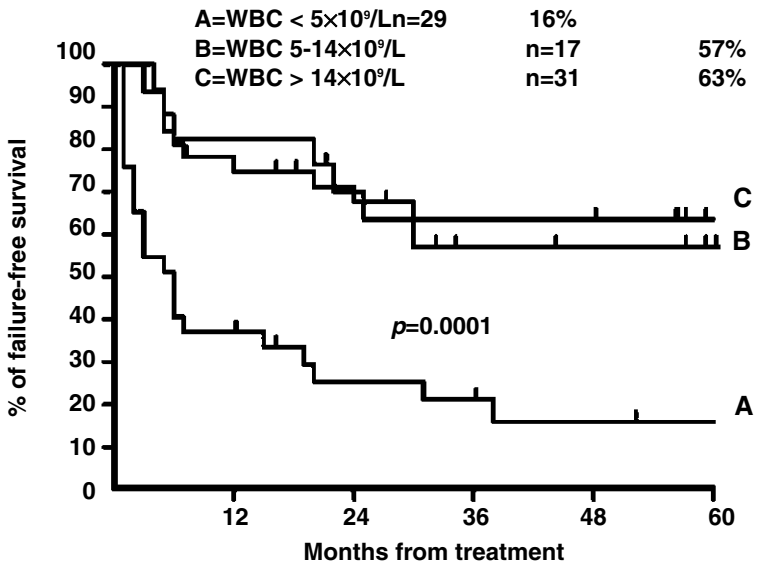

Figure 3. Actuarial failure-free survival in patients stratified according to the maximum white blood cell (WBC) achieved during treatment with G-CSF. Group A= maximum WBC counts <5×10⁹/L; Group B= maximum WBC counts 5-14×10⁹/L; Group C= maximum WBC counts >14×10⁹/L. Failure-free survival is significantly lower in patients who do not achieve a WBC count of 5×10⁹/L during treatment with the growth factor.

Predictive value of highest white blood cell count during G-CSF treatment

We have previously shown that achieving a white blood cell (WBC) count of 5 or 14×10⁹/L is predictive of outcome. We tested the effect of WBC levels on three different negative outcomes: lack of response, overall failure and death (Table 3). In both treatment groups, patients achieving a WBC greater than 14×10⁹/L were less likely to be non-responders, or failures or to be at risk of fatal complications. The differences were statistically significant in all subgroups. It is interesting to note that some patients (26%) receiving G-CSF 10 μg/kg/day still failed to respond despite a WBC greater than 14×10⁹/L, whereas in patients receiving G-CSF 5 μg/kg this was not the case. The actuarial survival rate at 5 years is 63% in patients with a highest WBC count (> 14×10⁹/L, 57%), in those whose WBC was 5-14×10⁹/L and 16% (in those whose highest WBC count never exceeded 5×10⁹/L) (p=0.0001).

Table 3. Highest white blood cell count during G-CSF treatment and outcome.

Treatment arm	*A 5 μg G-CSF*	*B 10 μg G-CSF*	*p*
Number of patients	38	39	
WBC×10⁹/L	NO response	NO response	
<5	50%	86%	
5-14:	25%	22%	
>14	0% p=0.001	26%	0.007
WBC×10⁹/L	Failure*	Failure	
<5	66%	87%	
5-14:	50%	33%	
>14	18% p=0.07	53%	0.003
WBC×10⁹/L	Death	Death	
<5	77%	77%	
5-14:	11%	8%	
>14	11% p=0.01	15%	0.002

**failure see text.*

Age effect

We found a strong age effect in this series of patients. Although the proportion of patients with very severe anemia was equally distributed in patients aged 0-20, 21-40 and over 40 years, survival was overall significantly lower in patients older than 40 years. The actuarial survival at 4 years was 81%, 80%, 34% for patients aged 0-20 (n=38), 21-40 (n=21), and over 40 (n=18) (p=0.0002) (Figure 3). In a multivariate Cox analysis including age, gender, neutrophil count at treatment, treatment arm, and highest WBC count achieved, significant predictive variables were WBC counts achieved (p=0.001) and age (p=0.03). We further investigated the correlation between age and maximum WBC increase and found a strongly significant inverse correlation: the older the patient, the lower the WBC increase (r=-0.40, p=0.0009). WBC counts up to <5, 5-14 and >14×10⁹/L were seen in 26%, 18% and 55% of patients, respectively, under 20 years; the corresponding figures were 33%, 28%, and 38% in the 21-40 year group and 66%, 22%, and 11% in patients over 40 years old (p=0.01).

Quality of response

Peripheral blood counts in partial responders were as follows : Hb 11.3 g/dL (range 7.5-15.2 g/dL), PMN 2.2 ×10⁹/L (range 0.8-14×10⁹/L), platelets 79×10⁹/L (range 29-164×10⁹/L) in patients in arm A, and Hb 9.5 g/dL (range 7.9-11.9 g/dL), PMN 2.5×10⁹/L (range 0.7-

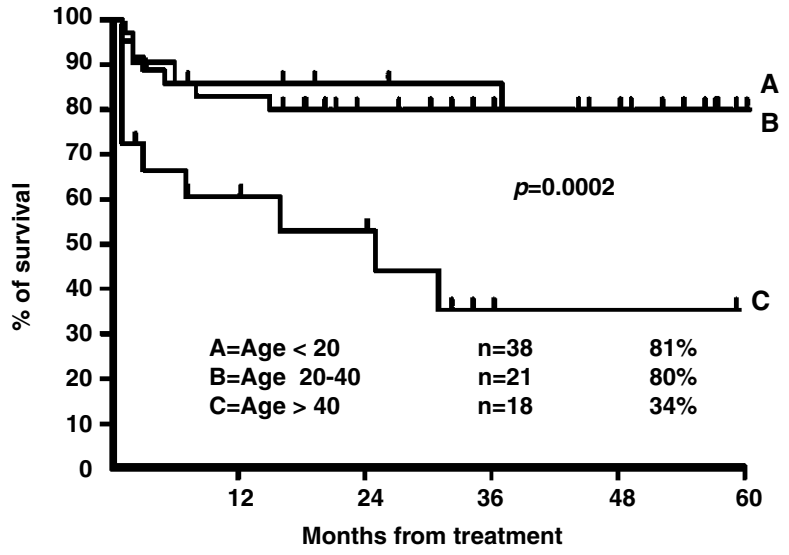

Figure. 4 Actuarial survival in patients stratified according to age. Group A= less than 20 years of age; Group B= 20-40 years of age; Group C= >40 years of age. FFS is significantly worse in patients above the age of 40.

6.9×10⁹/L), platelets 101×10⁹/L (range 55-147×10⁹/L) in arm B. Peripheral blood counts in complete responders were as follows: Hb 13.5 g/dL (range 10-16 g/dL), PMN 2.7×10⁹/L (range 1.5-24×10⁹/L), platelets 148×10⁹/L (range 102-239×10⁹/L) for patients in arm A, and Hb 12 g/dL (range 10-16 g/dL), PMN 2.5×10⁹/L (range 1.5-5.5×10⁹/L), platelets 157×10⁹/L (range 101-25×10⁹/L) for those in arm B.

Clonal disorders

Two patients in arm B developed acute myeloid leukemia and 2 patients in arm A developed cytogenetic clonal abnormalities (deletion 7).

Discussion

We tested the hypothesis that a higher dose of G-CSF would improve the response rate among SAA patients treated with antilymphocyte globulin and cyclosporin A. Secondary end-points that we examined were peripheral blood counts on day +120, early deaths and overall survival. There were significantly fewer hematologic responses in patients receiving G-CSF 10 μg/kg than in those receiving G-CSF 5 μg/kg: this was true at day +120, and was confirmed at the last follow-up. The numbers of complete responses were not significantly different in the two groups at day +120 (4 vs 9, p=0.1), and at the last follow-up (17 and 18, p=0.5). However the numbers of patients with partial remissions, that is patients achieving transfusion independence but not normal blood counts, were low in the G-CSF 10 μg/kg group at day +120 (22 vs 7, p=0.0004) and also at last follow-up (12 vs 2, p=0.002). This trial suggests that a higher dose of G-CSF, given for 30 consecutive days, does not improve trilineage response in aplastic anemia. This was further proven by comparing peripheral blood counts on day +120, as an objective way of evaluating hematologic response in patients with pancytopenia receiving treatment aimed at improving their blood counts. Hemoglobin, neutrophil and platelet counts on day +120 were overall not improved in patients receiving G-CSF 10 μg/kg, and, if anything, counts tended to be higher in the group treated with G-CSF 5 μg/kg. This finding suggests that a higher dose of G-CSF does not modify the kinetics of hematologic recovery in SAA patients treated with immunosuppressive therapy.

Secondary end-points of this study included analysis of early deaths and survival, and again we could not prove that a higher dose of G-CSF reduced early infection-related deaths. The survival rate at last follow-up was 72% among patients receiving G-CSG 5 μg/kg and 67% among those who received 10 μg/kg. We did confirm an effect of maximum WBC increase during G-CSF treatment on outcome: in a previous study on 100 patients receiving ALG, CyA and G-CSF 5 μg/kg, the median maximum WBC count was 14×10⁹/L and the 25th percentile was 5×10⁹/L.[11] We showed that patients not achieving a WBC of 5×10⁹/L had a significantly greater risk of not responding and of death than did patients with achieving WBC counts of 6-14 or >14×10⁹/L.[11] In the present study we confirmed these results using the same WBC cut-off values of 5 and 14×10⁹/L: patients not achieving a WBC of 5×10⁹/L had a 72% probability of not responding, a 79% probability of failing primary therapy and a 59% risk of death. These figures were 13%, 35% and 10% for patients achieving a WBC of >14×10⁹/L during G-CSF treatment. This held true for patients treated with either the 5 or 10 μg/kg regimen. In a Cox multivariate analysis, maximum achieved WBC count and patient's age were the two factors predicting survival. Indeed we found a significant effect of age in

this group of patients, the actuarial survival at 4 years being 81%, 79%, 34% for patients aged 0-20, 21-40 and over 40 years old. Because the severity of the aplasia, as indicated by initial neutrophil counts, was comparable in the three age groups, we then looked at whether WBC increase in response to G-CSF administration differed according to age. We identified a significant inverse correlation between age and WBC increase: the median WBC increase was 18.2, 11.4 and 8.7×10^9/L in patients aged 0-20, 21-40 and over 40 years old, respectively. Therefore older patients have lower WBC peak counts in response to growth factors, and have a higher probability of failing immunosuppressive therapy. The latter observation is in keeping with the results of a large study by the European Group for Blood and Marrow Transplantation (EBMT): the actuarial survival 5 years after immunosuppressive therapy was 72% in patients aged 20-49 years, 57% in patients aged 50-59 and 50% in patients aged 60 years or older.[17] The age effect was seen in one study from the National Institute of Health (NIH, USA) on 122 patients[18] and was not seen in another study of 84 patients by the German cooperative group.[19]

Clonal disorders have been a major concern in patients with SAA after the demonstration that these patients had a higher risk of developing myelodysplasia and leukemia if treated with immunosuppressive therapy than if treated with transplantation.[20] The concern was further enhanced by reports of a high incidence of clonal disorders in patients receiving prolonged therapy with G-CSF and cyclosporin therapy.[21, 22] Two recent papers seem to disprove that G-CSF is an additional factor exposing patients to late clonal disorders.[12,13] A prospective trial assigned aplastic anemia patients to receive or not to receive 90 days of G-CSF treatment, together with ALG/CyA and followed the two groups of patients for over 6 years:[13] no increased risk of leukemia/myelodysplasia was found in patients assigned to receive G-CSF. Our study appears to confirm these results, since 2 events occurred in each of the two treatment arms. We found a strong age effect in our study, which correlates with the inability of older patients to respond to growth factors given together with immunosuppressive therapy: increasing the dose of G-CSF from 5 to 10 μg/kg did not improve WBC increments nor did it improve response rates. In fact, patients receiving a higher dose of G-CSF had significantly fewer responses and worse failure free survival, and this was true in all three age groups. Based on these results we would not recommend the use of G-CSF 10 μg/kg as an adjunct to immunosuppressive therapy in SAA patients.

This trial highlights the uncertainties in the use of growth factors for patients with marrow failure: at present we would urge Centers to enter patients in the ongoing EBMT randomized trial comparing ALG, CyA with or without G-CSF 5 μg/kg. The expected 360 patients who will be enrolled in this study may give us a definitive answer on the place of G-CSF in the management of this rare and complex hematologic disorder.

AL and AB: conception and design, analysis and interpretation of data; drafting the article and final approval of the version sent for publication; BB: statistical analysis and interpretation of the data, final approval of the version sent for publication; AR, PS, CD, CF, SS, SV, WA, FL, DS participated in design process, analysis, critically revising for important intellectual content and final approval of the manuscript. The authors reported no potential conflicts of interest.

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Appendix

Participating centers:

Ancona, Università Cattedra Ematologia (P. Leoni); Bari, Clinica Pediatrica (D. De Mattia); Bergamo, Ospedale Civile, Divisione Ematologia (B. Comotti); Bologna, Università Cattedra Ematologia (C. Finetti); Bologna, Clinica Pediatrica III, Università (P. Rosito); Bolzano, Ospedale Divisione Ematologia (P. Coser); Brescia, Ospedale Divisione Ematologia (T. Izzi); Brescia, Universita', Clinica Pediatrica I (F. Porta); Cagliari, Ospedale Businco, Divisione Ematologia (G. Broccia); Cuneo, Ospedale Civile, Divisione Ematologia (A. Gallamini) ; Firenze, Clinica Pediatrica (A. Lippi); Genova, Istituto G. Gaslini, Divisione Medicina IV (C. Dufour); Genova, Ospedale San Martino, Divisione Ematologia II (MT. VanLint, A. Bacigalupo); Genova, Universita', Cattedra Ematologia (M Gobbi) ; Milano, Universita', Ist. Scienze Biomediche (P. Foa); Milano, Ospedale San Raffaele, Monza, Ospedale Div Ematologia (E. Pogliani); Napoli, Nuovo Policlinico, Divisione Pediatria (L. Pinto); Napoli, Universita', Cattedra Ematologia (B. Rotoli); Nuoro, Ospedale Civile, Divisione Ematologia (A. Gabbas); Palermo, Ospedale Cervello, Divisione Ematologia, Parma, Azienda Ospedaliera Ematologia/Oncologia Pediatrica (G. Izzi); Pavia, Policlinico S. Matteo, Divisione Pediatria I (F. Locatelli); Pavia, Universita', Cattedra Ematologia (P. Alessandrino); Pescara, Ospedale, Div. Ematologia (P. Di Bartolomeo); Pisa, Ospedale Unità Operativa Ematologia (F. Caracciolo); Reggio Calabria, Ospedali Riuniti, Dipartimento di Ematologia (P. Iacopino); Roma, Università "La Sapienza", Divisione Ematologia (W. Arcese); Roma, Ospedale Bambin Gesu' (G. De Rossi); Roma, Ospedale San Camillo, Div. Ematologia (A Locasciulli); S Giovanni Rotondo, Casa Sollievo Sofferenza, Divisione Ematologia; Torino, Clinica Pediatrica, Divisione Ematologia (P. Saracco); Udine, Universita', Cattedra Ematologia; Verona, Policlinico Borgoroma, Divisione Ematologia (G. Todeschini); Vicenza, Ospedale, Divisione Ematologia (E. Di Bona).

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