Antilymphocyte Globulin, Cyclosporin, and Granulocyte Colony-Stimulating Factor in Patients With Acquired Severe Aplastic Anemia (SAA): A Pilot Study of the EBMT SAA Working Party


Patients with severe aplastic anemia (SAA) and a neutrophil (PMN) count of less than 0.5 × 10^9/L are exposed to a high risk of early mortality when treated with antilymphocyte globulin (ALG) and steroids, with the major problem being infectious complications. The addition of human recombinant granulocyte colony-stimulating factor (rhG-CSF) to ALG may reduce early mortality by improving neutrophil counts in the short term. To test the feasibility of this approach, the SAA Working Party of the European Group for Blood and Marrow Transplantation (EBMT) designed a pilot study that included rhG-CSF (5 μg/kg/d, days 1 through 90), horse ALG (HALG; 15 mg/kg/d, days 1 through 5), methylprednisolone (2 mg/kg/d, days 1 through 5, then tapering the dose), and cyclosporin A (CyA; 5 mg/kg/d orally, days 1 through 180). Patients with newly diagnosed acquired SAA (untreated) and with neutrophil counts of ≤0.5 × 10^9/L were eligible. Forty consecutive patients entered this study and are evaluable with a minimum follow up of 120 days: the median age was 16 years (range, 2 to 72 years), the interval between diagnosis to treatment was 24 days, and the median PMN count was 0.19 × 10^9/L. Twenty-one patients had hemorrhages, and 19 were infected at the time of treatment. Overall, treatment was well tolerated: the median maximum PMN count during rhG-CSF administration was 12 × 10^9/L (range, 0.4 × 10^9/L to 44 × 10^9/L). There were three early deaths (8%) due to infection. Four patients (10%) showed no recovery, whereas 33 patients (82%) had trilineage hematologic reconstitution and became transfusion-independent at a median interval of 115 days from treatment. Median follow up for surviving patients is 428 days (range, 122 to 1,005). Actuarial survival is 92%; 86% and 100% for patients with PMN counts less than 0.2 × 10^9/L or between 0.2 × 10^9/L and 0.5 × 10^9/L, respectively. This study suggests that the addition of rhG-CSF to ALG and CyA is well tolerated, is associated with a low risk of mortality, and offers a good chance of hematologic response. This protocol would appear to be an interesting alternative treatment for SAA patients with a low PMN count who lack an HLA-identical sibling.

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**MATERIALS AND METHODS**

**Trial design.** This trial was designed by the SAA Working Party of the EBMT. Patients with newly diagnosed (within 30 days) acquired SAA without concomitant or preceding neoplasia and aged 1 to 70 years were eligible. All patients had anemia requiring red blood cell support, thrombocytopenia (platelet count less than 30 \(\times 10^9\)/L), neutropenia (PMN count <0.5 \(\times 10^9\)/L), and hypoaplastic marrow with less than 5% blasts, as indicated by marrow histology.

**Treatment schedule.** Horse ALG (HALG; Lymphoglobulin, Meirieux, Lyon, France), 15 mg/kg of body weight/d on days 1 through 5, was given as a slow intravenous infusion in saline over 6 to 8 hours after premedication with antihistamines, together with methylprednisolone (MPred), 2 mg/kg/d IV on days 1 through 5, 1 mg/kg/d on days 6 through 10, and then tapering the dose and stopping on day +30. CyA, 5 mg/kg orally, was started on day +1 and continued at least until day +180. All patients also received rhG-CSF, 5 \(\mu\)g/kg/d subcutaneously (SC) from day +1 to day +90: four patients received lenograstim (Chugai Rhone Poulenc, Paris, France), and 36 patients received filgrastim (Amgen Roche). CyA was reduced in case of increasing creatinine or bilirubin levels. Gut decontamination with nonabsorbable antibiotics was recommended, as well as hospitalization in single rooms with mask protection. Patients not responding at 120 days, as indicated by persisting requirement of blood transfusions and pancytopenia, were either offered marrow transplantation, if a suitable donor was available, or a second course of rabbit ALG (RALG Thymoglobulin; Meirieux), 3.5 mg/kg of body weight/d on days 1 through 5; together with MPred, 2 mg/kg/d for 5 days and then halving the dose every 5 days: CyA, 5 mg/kg/d orally; and rhG-CSF, 5 \(\mu\)g/kg/d starting on day 1 of RALG treatment.

Patients were observed for an additional 120 days and assessed for clinical response.

**Patients.** Between October 1, 1991, and December 31, 1993, 40 patients were treated and are evaluable. Clinical details of the patients are outlined in Table 1.

**Response.** Patients were classified as follows: complete responders: transfusion-independent, with a hemoglobin level of \(\geq 11\) g/dL, a neutrophil count greater than 1.5 \(\times 10^9\)/L, and a platelet count greater than 100 \(\times 10^9\)/L; partial responders: transfusion-independent with a hemoglobin level of \(\geq 8\) g/dL, a neutrophil count greater than 0.5 \(\times 10^9\)/L, and a platelet count greater than 20 \(\times 10^9\)/L. Persistence of transfusion requirement was evidence of no response.

**Statistical analysis.** All eligible patients, including those who died within day +100 from therapy, were included in the analysis. Survival curves were calculated by the method of Kaplan and Meier. Data were also analyzed by contingency tables, log rank, rank sum Mann-Whitney, and Fisher tests.

**RESULTS**

**Tolerance and side effects.** Overall, treatment was very well tolerated: there were no serious anaphylactic reactions requiring discontinuation of ALG or rhG-CSF. Subcutaneous G-CSF was given without major hemorrhagic or infectious complications at the site of injection. Increments in white blood cell (WBC) counts up to 44 \(\times 10^9\)/L were tolerated without pain. Most patients showed increased counts during the first month of treatment, with a median maximum WBC count of 12 \(\times 10^9\)/L (range, 0.4 \(\times 10^9\)/L to 44 \(\times 10^9\)/L).

**Early mortality.** Of 40 patients, three (8%) died of sepsis within 100 days: they were all in the group with less than 0.2 \(\times 10^9\)/L PMNs (Fig 1), were 49, 47, and 16 years old, and had less than 1 \(\times 10^9\)/L total WBCs at the time of death.

**Response.** Four patients (10%) remained transfusion-dependent or showed minimal improvement of blood cell counts and were considered nonresponders [two underwent allogeneic bone marrow transplantation (BMT)], whereas 33 patients (82%) became transfusion-independent (Fig 1). The proportion of responders with neutrophil counts less than versus \(>0.2 \times 10^9\)/L was 15 of 21 (71%) versus 18 of 19 (95%), respectively (\(P = .06\), exact Fisher’s test). Responses were equally distributed when patients were stratified for age, sex, and interval between diagnosis and treatment (Table 2). However, there were significantly less responders among patients not achieving a total leucocyte count of 5 \(\times 10^9\)/L during rhG-CSF treatment (Table 2).

**Quality and timing of response.** Sixteen patients (40%) were considered partial responders because of improved peripheral blood cell counts without transfusion support, and 17 patients (42%) showed a complete response with normalization of peripheral blood cell counts (Fig 1). Peripheral blood cell counts are outlined in Table 3. When analyzing the time interval between treatment and the last transfusion of red blood cells or platelets, the 25th, 50th, and 75th percentiles were, respectively, 61, 115, and 247 days. Complete responders stopped requiring transfusion support significantly earlier than partial responders (median, 65 vs 215 days; \(P = .006\) by Student’s t-test; Table 3). The overall actuarial probability of discontinuing transfusion support is 97% at 18 months (Fig 2).

**Number of treatment courses.** Of the 17 complete responders, only one required two courses of ALG compared with six second ALG courses in 16 partial responders (\(P = .03\) by Fisher’s test; Table 3). Of the four nonresponders, one received two courses of ALG, and two were transplanted.

**Relapse and clonal disease.** One patient had a relapse at discontinuation of CyA on day +180 and responded again to the administration of CyA + rhG-CSF. We have since then continued CyA in responders beyond 180 days with slow tapering. No patient has developed a clonal disease.

**Survival.** Median follow-up for surviving patients is 428 days (range, 122 to 1,005 days). Actuarial survival is 92% (Fig 3): 86% and 100% for patients with PMN counts of less than 0.2 \(\times 10^9\)/L and between 0.2 \(\times 10^9\)/L and 0.5 \(\times 10^9\)/L, respectively.
Survival was not influenced by sex (95% vs 89% for males and females, respectively; \( P = .3 \)), age (95% vs 89% for age less than 16 years and \( \geq 16 \) years, respectively; \( P = .4 \)), or interval between diagnosis and treatment (93% vs 68% for less than 30 days and \( \geq 30 \) days, respectively; \( P = .1 \)). Survival of 10 patients who did not achieve an increase of their total WBC count to \( 5 \times 10^9/L \) was significantly lower than survival of 30 patients showing WBC increments \( \geq 5 \times 10^9/L \) (70% vs 100%, respectively; \( P = .003 \)).

Failures. There were three early deaths and four nonresponders: of the latter, two have undergone allogeneic BMT and are censored as surviving at the time of transplant (both survive), whereas two are alive with transfusion support.

DISCUSSION

The first aim of this study was to test the tolerance for combined ALG + CyA + rhG-CSF and MPred treatment in SAA patients with neutrophil counts \( \leq 0.5 \times 10^9/L \). There were no major side effects when these agents were given together: some patients complained of chills and fever during ALG administration, as previously described,\(^{17}\) without requiring discontinuation of ALG.

This protocol was associated with a very low rate of early and overall mortality, and one may ask whether this effect was due to the early use of rhG-CSF. In the recently published German trial,\(^6\) one arm included HALG (Merieux), CyA, and MPred as given in the present study, though HALG was given at the same daily dose but for 8 rather than 5 days. In that trial, 25 patients had neutrophil counts \( \leq 0.5 \times 10^9/L \). There were three early deaths within day 100, and 11 total deaths within day 1,524. When compared with this study, in which patients received a superimposable immunosuppressive regimen with the addition of rhG-CSF, there was no difference in the number of early deaths (12% vs 8%; \( P = .4 \)) or responders (68% vs 82%; \( P = .1 \)), and the speed of response seems unchanged. The significant difference was in the number of total deaths: 11 of 25 (44%) compared with

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\begin{align*}
\text{Table 2. Hematologic Response and Clinical Data} \\
\text{No. of patients} & \quad 7 & 33 & \text{Response} \\
M & 4 & 18 \\
F & 3 & 15 \\
\text{Neutrophil count} & \text{<0.2 \( \times 10^9/L \)} & 6 & 15 \\
& \text{0.2 \( \times 10^9/L \)} & 1 & 18 \\
\text{Interval from diagnosis to treatment} & \text{<30 d} & 5 & 23 \\
& \text{\geq 30 d} & 2 & 10 \\
\text{Highest WBC count} & \text{<5 \( \times 10^9/L \)} & 6 & 1 \\
& \text{\geq 5 \( \times 10^9/L \)} & 1 & 26 \\
\text{Age} & \text{<16 y} & 3 & 18 \\
& \text{\geq 16 y} & 4 & 15 \\
\end{align*}
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\* By Fisher’s test.

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\begin{align*}
\text{Table 3. Quality and Timing of Hematologic Recovery} \\
\text{in Responders} \\
\text{No. of patients} & \quad 33 & 16 & 17 \\
\text{Hemoglobin level} & (g/dL) & 11 (7-14.7) & 10 (8-12) & 12 (11-14.7) \\
\text{Neutrophils count} & (>10^9/L) & 2 (1-7) & 2 (1-7) & 2 (1.5-5.6) \\
\text{Platelet count} & (>10^9/L) & 94 (3-173) & 40 (20-92) & 132 (100-173) \\
\text{Interval from treatment to response} & \text{<30 d} & 93 (14-441) & 215 (61-441) & 65 (14-371) \\
\text{Second ALG treatment (no/yes)} & 32/8 & 10/6 & 16/1 \\
\end{align*}
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Data are median values (range) except for second ALG treatment.
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3 of 40 (8%; \( P = .0007 \), Fisher's test), possibly due to late failures and relapses in the German trial (Frickhofen et al. and Frickhofen, personal communication, April 1994).

Similar results were seen when looking at EBMT Registry data on 84 SAA patients with neutrophil counts of \( \leq 0.5 \times 10^9/\text{L} \), receiving ALG + CyA + prednisolone: there were eight early deaths (9%) and 41 total deaths (48%) (unpublished data, April 1994). The actuarial survival rate at 3 years in the EBMT Registry data is 68%, which is significantly lower than the 92% rate of the present study (\( P = .03 \)).

The number of patients who died in the present study is very small and makes the analysis of prognostic factors difficult. However, the three deaths were all in the group of patients with neutrophil counts of less than 0.2 \( \times 10^9/\text{L} \), and occurred in patients who did not improve their total WBC count to at least 5 \( \times 10^9/\text{L} \). Indeed, 10 patients were in this group, and three died of infection. It may be that a higher dose of rhG-CSF, such as 10 or 20 \( \mu \text{g/kg} \), as already tested in children with neutropenia, may further reduce the risk of early life-threatening infections.

However, the outcome for SAA patients is not only determined by improved neutrophil counts in the first months, but is also strongly associated with long-term hematologic recovery. In this study, the response rate was very high: of the initial 40 patients, 33 became independent from transfusion support by day 14 and day 404 posttreatment, with a median interval of 112 days. Therefore, the requirement of transfusions 4 months after initial treatment did not predict definite failure, as 50% of responses occurred beyond this time point, with increasing actuarial probability of response to 97% at 18 months. Slow responders often required a second course of immunosuppression and remained thrombocytopenic, with platelet counts in the range of 40 \( \times 10^9/\text{L} \). Despite these negative observations, slow responders have been reported to have a lower risk of relapse in the long term.14

The overall response rate at 1 year in this study (82%) is higher than the response rate in the German trial (68%) using ALG + CyA without rhG-CSF (\( P = .1 \)), and the number of deaths is significantly lower: this may suggest an advantage for patients receiving rhG-CSF due not to an early increase of neutrophil counts, but rather to the mobilization of hematopoietic progenitors.

Indeed, a number of these patients underwent weekly leukophereses while receiving rhG-CSF therapy:19 the number of hematopoietic progenitors recovered from the peripheral blood varied greatly from patient to patient, but in many cases, it was in the range described to allow engraftment in an autologous transplant setting, ie, over 2 \( \times 10^9/\text{kg} \). Further studies on the quality of hematopoietic progenitors have been performed, and preliminary results have been reported.20 The fact that hematopoietic progenitor cells can be found in the peripheral blood of SAA patients after ALG treatment is not a new finding: many years ago, Torok Storb et al.21 described an increased number of erythroid blast-forming units (BFU-E) after ALG treatment in some patients and observed an association with hematologic recovery. Several subsequent reports have indicated that ALG can directly or indirectly stimulate hematopoietic progenitors to proliferate and differentiate.22-27 Therefore, it may be that hematologic response of SAA patients is dependent on circulation in the peripheral blood and reseeding of hematopoietic progenitors: the use of rhG-CSF together with ALG, as described in this study, may simply amplify this phenomenon and possibly increase the chance of successful reseeding.

HLA-identical sibling transplantation remains the treatment of choice for young patients with very severe aplastic anemia,28 not necessarily because of significantly superior survival in the short term,29 but because of a lower risk of developing myelodysplasia or leukemia when compared with patients given ALG.30

Results of marrow transplantation from family-mis-
matched or unrelated-matched donors have been rather disappointing,² with overall survival well below 50% in most series. Until these results improve with better donor-recipient matching or with different transplant protocols, patients without an HLA-identical sibling and older patients may be considered eligible for the four-drug program described in this report.

APPENDIX

The following centers/investigators participated in this study: Clinica Pediatrica/D. De Mattia, Bari, Italy; Ospedale Civile, Divisione Ematologia/R. Bassan, Bergamo, Italy; Clinica Pediatrica III, Università/P. Rosito, Bologna, Italy; Università, Clinica Pediatrica IV/F. Porta, Brescia, Italy; Department of Pediatrics, Queen Fabiola Hospital/W. Buyan, Brussels, Belgium; Ospedale Businco, Divisione Ematologia/G. Broccia, Cagliari, Italy; St. James Hospital, Hematology Department/L. Fitzgerald, Dublin, Ireland; Ospedale Civile, Divisione Ematologia/A. Gallamini, Cuneo; Clinica Pediatrica/A. Lippi, Florence; Istituto G. Gaslini, Divisione Medicina IV/P.G. Mori, Genova; Ospedale San Martino, Divisione Ematologia II/A. Bacigalupo, Genova; Nuevo Policlinico, Divisone Pediatrica/L. Pinto, Naples; Ospedale Civile, Divisione Ematologia/A. Gabbas, Nuoro; Ospedale Cervello, Divisione Ematologia/I. Majolino, Palermo; Policlinico S. Matteo, Divisione Pediatrica/IF. Locatelli, Pavia; Ospedale Riuniti; Divisione Ematologia/P. Jacopino, Reggio Calabria; Universita "La Sapienza"; Divisione Ematologia/W. Arcese, Rome; Casa Sollievo della Sofferenza, Divisione Ematologia/M. Carotenuto, S.G. Rotondo; Ospedale Molinette, Divisione Ematologia/P. Saracco, Torino;¹ and Policlinico Borgoroman, Divisione Ematologia/G. Todeschini, Verona, Italy.

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