Immunosuppressive Therapy of Aplastic Anemia: Results of a Prospective, Randomized Trial of Antithymocyte Globulin (ATG), Methylprednisolone, and Oxymetholone to ATG, Very High-Dose Methylprednisolone, and Oxymetholone

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Sixty-eight patients with moderate (n = 15) or severe (n = 53) aplastic anemia were entered into a prospective, randomized, two-arm treatment study comparing antithymocyte globulin (ATG), lower-dose methylprednisolone (LDM) and oxymetholone to ATG, higher-dose methylprednisolone (HDM), and oxymetholone. There were no differences between the two groups when comparing age, sex, etiology of aplasia, disease duration, severity of aplasia, or pretherapy granulocyte counts. Side effects of LDM and HDM were similar. Of the 64 patients evaluable for response to therapy, 12 of 33 (36%) who received LDM had complete, partial, or minimal responses compared with 15 of 31 patients (48%) who received HDM (P = .33). Actuarial survival at 4 years is 43% for patients in the LDM group and 47% for patients in the HDM group (P = .99). Causes of death included hemorrhage, infection, evolution to acute leukemia, and complications of subsequent bone marrow transplantation. Long-term complications included paroxysmal nocturnal hemoglobinuria (n = 3), evolution to myelodysplasia or acute leukemia (n = 6), and recurrent aplasia (n = 6). We were unable to show a significant difference in toxicity, response rate, or survival for patients treated with ATG, oxymetholone, and LDM compared with patients who received ATG, oxymetholone, and HDM. © 1992 by The American Society of Hematology.

Materials and Methods

Patients of any age who had a diagnosis of moderate or severe acquired aplastic anemia were eligible for study. Between September 1984 and September 1988, 68 patients ranging from 1 to 74 years of age were treated. Fifty-three patients had severe aplasia as defined by the International Aplastic Anemia Study Group and 15 had moderate disease. All patients had their diagnoses confirmed by bone marrow aspirates and biopsies before therapy. Cytogenetic studies were requested on all patients and marrow samples from 58 patients were submitted for evaluation. Forty-five patients had ≥ 5 metaphases analyzed and 44 had normal karyotypes. One patient had both a missing Y chromosome and an additional chromosome in two of 50 metaphases examined. The latter patient was treated before results of the cytogenetic studies were made available. In 13 cases, cytogenetic studies were inadequate (0 to 4 metaphases examined). None of the patients were candidates for marrow transplants as primary therapy, either because of age (over 40 years old) or because an appropriate donor was not available. Informed consent was obtained before therapy using forms approved by the Institutional Review Board of the Hutchinson Cancer Research Center. Pretreatment data are summarized in Table 1.

All patients were treated in private, nonsterile rooms. Patients with granulocyte counts less than 500/mm³ who developed a temperature greater than 38.5°C were treated empirically with broad spectrum antibiotics. No patient received prophylactic granulocyte transfusions. Red blood cell (RBC) transfusions were administered when the hematocrit was ≤ 25%, and platelet transfusions were administered when the platelet count was ≤ 20,000/ mm³. No patient underwent splenectomy before receiving immunosuppressive therapy.

The treatment schedule was as follows: ATG (ATGAM; Upjohn Co, Kalamazoo, MI), 15 mg IgG/kg body weight was administered intravenously (IV) daily for 10 days. Day 1 was defined as the day ATG therapy began. Before the first dose, all patients received intradermal tests with a 1:1,000 dilution of ATG. Each daily dose of ATG was administered over 6 to 12 hours, as tolerated. Multiple lots of ATGAM were used during the course of the study. Patients were randomized to receive either LDM or HDM. Those random-
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Each daily dose of LDM received 0.5 mg/kg methylprednisolone IV before oral/d was administered. IV doses of methylprednisolone were begun on day 1 and infused over 12 hours (maximum dose, 500 mg); days 9 to 25 of oral/d were administered. IV doses of methylprednisolone were tapered over 4 months unless toxicity necessitated early discontinuation. In both treatment groups oxymetholone was begun on day 12 at a dose of 3 mg/kg orally/d and then continued for at least 3 months unless toxicity necessitated early discontinuation. For patients who remained severely aplastic after completion of their IV methylprednisolone therapy when they were clinically stable. The median duration of hospitalization was 18 days for each treatment group.

Response to therapy was initially evaluated on day 75. Patients who showed no evidence of improvement in peripheral counts at that time and patients who had died were defined as nonresponders. For patients considered responders, the degree of response was assessed when the maximum improvement in peripheral counts occurred. Complete responders attained a normal hematocrit, a granulocyte count $\geq 1,000/mm^3$, and a platelet count of $\geq 100,000/mm^3$ without transfusions. Partial responders had improvement in all three cell lines, with granulocyte counts $\geq 500/mm^3$, an absence of infections, and no transfusion requirement. Minimal improvement was defined as an increase in granulocyte counts of $\geq 500/mm^3$, but transfusions of RBCs and platelets were still required.

Four patients were not considered evaluable for response: the two patients who received alternative immunosuppressive regimens, one patient who developed leukemia on day 16, and one patient who received additional immunosuppressive therapy with cyclosporine.

Patients who were defined as nonresponders were eligible to receive secondary therapy with additional immunosuppression or supportive care at the discretion of their referring physicians. Patients for whom a suitable unrelated or mismatched family member marrow donor was identified were offered a marrow transplant as secondary therapy.

**Statistical methods.** Differences in response to therapy were assessed using a Pearson $\chi^2$ statistic. Survival probabilities were estimated using the Kaplan-Meier method and comparisons were based on the log-rank statistic. Cumulative incidence estimators of recurrence of aplasia and of hematologic malignancy were calculated. Median follow-up among surviving patients is 4.8 years (range, 1.8 to 6.3 years). All P values are two-sided.

**RESULTS**

As seen in Table 1, there were no differences between the two treatment groups when comparing patient sex, age, etiology of aplasia, duration of aplasia, prior therapy, severity of disease, or pretreatment granulocyte count.

**Toxicity.** Side effects associated with LDM and HDM were similar and are summarized in Table 2. There was no difference in the incidence of aseptic necrosis after therapy in either treatment group. Two patients, both of whom were treated with LDM, had preexisting aseptic necrosis. Clinical manifestations of serum sickness, including fever, rash, and arthralgias, also were not different between treatment groups and were noted in 16 patients who received LDM and in 12 patients who received HDM.

**Response to therapy.** As shown in Table 3, 12 of the 33 (36%) evaluable patients who received LDM had a complete response.
were also comparable, with no increase in fatal infections
with 4 of 8 (50%) receiving both treatment groups, 43% for patients who received aplastic anemia.

Noted in the higher dose steroid group compared with the lower dose group (Table 3).

Excluding the four patients who were placed on LDM, 10 of 27 (37%) who received severe aplasia, 10 of 27 (37%) who received moderate AA, and 5 of 20 (25%) who received severe AA (N = 15) responded compared with 11 of 23 (48%) who received HDM, 5 of 20 (25%) who received moderate AA (N = 15) of patients alive

Survival. Actuarial survival at 4 years is comparable for both treatment groups, 43% for patients who received LDM and 47% for patients who received HDM (P = .33). Excluding the four patients who were placed on LDM, 11 of 29 (38%) responded (P = .41). Table 4 summarizes the responses observed according to severity of disease and treatment group. Among the 50 evaluable patients who had severe aplasia, 10 of 27 (37%) who received LDM responded compared with 11 of 23 (48%) who received HDM (P = .44). For the 14 evaluable patients with moderate disease, 2 of 6 (33%) receiving LDM responded compared with 4 of 8 (50%) receiving HDM.

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had a second relapse requiring tertiary therapy (ATG, one patient; GM-CSF, one patient). Four of the six patients are alive and two have died, one from complications of a marrow transplant and one after failing tertiary therapy with GM-CSF.

Six patients have had evolution to a hematologic malignancy. Five of the six were evaluable for response to immunosuppressive therapy; one patient had a partial response, two had minimal improvement, and two were nonresponders. Median time to diagnosis was 8.5 months (range, 0.5 to 15 months) after initiating ATG therapy. Two patients developed a myelodysplastic syndrome (MDS) as diagnosed by marrow morphology alone in one case and as suggested by identification of a clonal cytogenetic abnormality [13] [q13q31] in the second case. Four patients developed acute leukemia; two had erythroleukemia and two did not have their leukemia classified before death. A diagnosis of MDS was considered in three of these six patients before entry into this study; however, marrow morphology was not considered sufficiently abnormal to exclude a diagnosis of aplastic anemia. Only the patient with MDS associated with deletion of chromosome 13 is currently surviving. Cytogenetic data were not available on this patient before therapy.

Figure 2 illustrates the probability of developing either a hematologic malignancy or recurrent aplasia for all patients treated on this study. At 4 years this probability is 18%. As shown, the incidence of either long-term complication is 9%.

Bone marrow transplantation was attempted in nine patients who failed their initial immunosuppressive therapy (n = 7), who relapsed after an initial response (n = 1), or who developed MDS (n = 1). Marrow donors included HLA-identical siblings (n = 2), a one HLA-antigen mismatched uncle (n = 1), and HLA-matched unrelated donors (n = 6). Median time between primary immunosuppressive therapy and transplant was 7.6 months (range, 4.3 to 26 months). Eight of the nine patients underwent transplants at our center and, currently, only one patient is surviving 3.7 years after transplant. The surviving patient received marrow from an unrelated donor. The other eight patients died 9 to 139 days (median, 49.5 days) after marrow grafting. Causes of death included infection (n = 5), hemorrhage (n = 1), chronic graft-versus-host disease (n = 1), and development of a lymphoproliferative malignancy (n = 1).

DISCUSSION

For patients with aplastic anemia who are not eligible for a transplant from an HLA-identical sibling, immunosuppressive therapy has become the treatment of choice. Therapy with a single immunosuppressive agent has largely been replaced by combination therapy. ATG or ALG is usually included in the treatment regimen along with androgenic steroids, corticosteroids, and, most recently, cyclosporine. HLA haplotype-mismatched marrow infusion is no longer incorporated in such regimens because no significant improvement in response rates or long-term survival has been shown.14 The use of androgenic steroids remains controversial with mixed reports of its effectiveness when used in conjunction with ATG or ALG.5,15,16

Several nonrandomized trials have reported combination therapy with HDM, ALG, or ATG, with or without androgens as effective primary treatment of aplastic anemia or as salvage therapy for patients who failed their initial immunosuppressive regimen. Reported response rates have ranged from 27 to 67%.2,3,13 We initially studied the use of ATG, HDM, and oxymetholone in a nonrandomized study of 46 patients with moderate (n = 12) or severe (n = 34) aplastic anemia.6 Fifty-two percent of patients had a complete, partial, or minimal response. Actuarial survival at 4 years was 67% (95% CI = 52% to 79%). Despite the use of HDM, 50% of patients still developed signs or symptoms of serum sickness.

Figure 3 compares the updated actuarial survival for this nonrandomized trial with that of the current study. Survival at 4 years for all patients treated in the randomized study is 45% (95% CI = 33% to 56%). The difference in survival between these two curves is significant (P = .03). The increased early mortality noted in the randomized trial is not readily explained. Patient demographic data, risk factors associated with response and survival, and the level of supportive care patients received were similar in both studies. Acute toxicity of the treatment regimens was also similar. Among patients who died within the first 1.5 years, there was an increased number of deaths associated with infection in the current study (12 of 68 patients) compared with our previous trial (3 of 47 patients) (P = .998). This in part may reflect the poorer clinical condition of patients currently being sent to referral centers for treatment.

Also shown in Fig 3 is the survival curve for 70 patients with acquired aplastic anemia who received HLA-identical sibling donor transplants in Seattle during the time period of the two studies of immunosuppressive therapy (IST) (1983 to 1988). This curve is significantly different from the survival curve for the combined data of the current randomized IST study (P = .002); however, it is not different from
the results of our prior nonrandomized IST study (P = .147). Because of the age restriction for marrow transplantation, all patients in the transplant group were less than 50 years old. Although late deaths, usually associated with chronic graft-versus-host disease, occurred in the transplant group, this survival curve reaches a plateau at 3 years. In contrast, a plateau is not evident in either curve of patients treated with IST.

Several groups have published long-term follow-up data on patients with aplastic anemia treated with immunosuppressive therapy. Recurrent aplasia, PNH, MDS, and acute leukemia have all been recognized in this patient population. The actuarial probability of developing PNH, MDS, or acute leukemia has been reported to be as high as 57% 8 years after immunosuppressive therapy. In our current study, PNH has become clinically evident in only three patients, all of whom were treated in the HDM group. Because patients are not routinely tested for laboratory evidence of PNH after returning home, and follow-up is still relatively short, the actual incidence of PNH in our population is uncertain. In another single center study, Marsh et al reported similar results with hemolytic PNH developing in two of 64 patients with aplasia treated with an immunosuppressive regimen.

The incidence of developing a hematologic malignancy in the current series (6 of 68) is similar to that previously reported by the group in Basel (8 of 103). In contrast is the report from the Hammersmith Hospital; with a similar length of follow-up, only 1 of 64 patients developed a T-cell lymphoma and none developed MDS or acute leukemia. Differences between reported studies may be explained in part by differences in long-term survival with fewer patients at risk in studies reporting lower response rates and increased early mortality. In addition, inclusion criteria for patient entry into such studies may differ, eg, we chose to treat three patients in whom the diagnosis of myelodysplasia was questioned on at least one marrow exam, but who had normal cytogenetics. The difficulty in making such a diagnosis solely on the basis of marrow morphology emphasizes the importance of obtaining cytogenetic analyses pretherapy. Despite the hypocellularity of these patients' marrows, we were successful in obtaining adequate cytogenetic information in 76% of specimens received.

Nine patients in the current study who failed their initial course of immunosuppression or who evolved to MDS received a marrow transplant. As noted, most patients died early after marrow grafting from complications associated with their prolonged pancytopenia, ie, hemorrhage or infection. For patients without suitable related donors, unrelated donor searches generally were begun coincident with initiation of immunosuppressive therapy. Despite this attempt to minimize the time from initial therapy to transplantation, the clinical condition of the patients often deteriorated. As the time needed to identify suitable HLA-identical unrelated donors decreases, these results may improve. The ultimate role of unrelated or mismatched donor transplants for patients who fail or relapse after IST therapy remains to be defined. Other groups have reported successful salvage therapy for this group of patients using sequential courses of immunosuppression.

Given the sample size in this clinical trial, the statistical power of the analysis is limited. The 95% confidence interval for the difference between the response rates in the two treatment groups is 12% to 36%. Although we have not shown a significant benefit of HDM added to ATG and oxymetholone therapy, it is possible that a much larger study might detect a therapeutic advantage. However, with the commercial availability of ATG and ALG preparations such a large study would only be feasible in a multicenter trial. However, at this time, other agents, including cyclosporine and recombinant hematopoietic growth factors, are currently the major focus of investigative interest.

In this study HDM, ATG, and oxymetholone were not associated with a statistically significant improvement in response rate or survival compared with LDM, ATG, and oxymetholone. HDM also did not protect patients from the side effects of ATG administration. Conversely, we did not detect any increase in adverse effects associated with the use of HDM. Given the similar outcomes between the two groups, this study has found no reason to recommend the routine use of HDM along with ATG for treatment of aplastic anemia.

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