Marrow Transplantation for Severe Aplastic Anemia: Methotrexate Alone Compared With a Combination of Methotrexate and Cyclosporine for Prevention of Acute Graft-Versus-Host Disease


Forty-six patients with severe aplastic anemia (median age, 23 years) were treated with high-dose cyclophosphamide followed by infusion of marrow from an HLA-identical family member. To evaluate postgrafting prophylaxis for graft-versus-host disease (GVHD), they were entered into a prospective randomized trial comparing the effect of a combination of methotrexate and cyclosporine (n = 22) to that of methotrexate alone (n = 24). Forty-four of the forty-six patients had evidence of sustained marrow engraftment. Only one patient in each of the two study groups showed graft rejection. A significant reduction in the cumulative incidence of grades II to IV acute GVHD was seen in patients given methotrexate/cyclosporine (18%) compared with those given methotrexate alone (53%) (P = .012). In three patients given methotrexate alone, grade III developed, and in six, grade IV acute GVHD developed, compared with none given methotrexate/cyclosporine. Eighteen of the 22 patients given methotrexate/cyclosporine and 15 of the 24 given methotrexate alone are alive between 5.5 and 44.5 months (median, 18 months), with actuarial survival rates at 2 years of 82% and 60%, respectively (P = .062). The incidence of fatal infections was higher in patients given methotrexate alone, whereas there are as yet no significant differences in the incidence of chronic GVHD. We conclude that methotrexate/cyclosporine treatment resulted in a significant decrease in the incidence and severity of acute GVHD in patients who received transplants for severe aplastic anemia and thus an improvement in survival.

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be obtained; the patients' records merely indicated that multiple transfusions had been administered.

Only three patients given methotrexate/cyclosporine and one given methotrexate alone had a positive relative response of more than 2.6%. In one patient given methotrexate, the relative response was not evaluable.

Patients randomized to receive methotrexate alone were given methotrexate, 15 mg/m² IV on day 1 and 10 mg/m² on days 3, 6, and 11 postgrafting. Reductions or omissions of methotrexate doses were common and were due to renal insufficiency, marrow toxicity, or liver function abnormalities associated with severe GVHD. Cyclosporine treatment was begun on the day before transplantation and was given IV at 1.5 mg/kg every 12 hours until recovery from chemotherapy-induced gastrointestinal toxicity. At that time, patients were given cyclosporine orally at 6.25 mg/kg every 12 hours. The full dose of cyclosporine was given until day 50 unless nephrotoxicity developed; the dose was reduced by 50% if the serum creatinine level doubled above baseline values and was temporarily withheld if the creatinine value exceeded 2 mg/dL. After day 50 cyclosporine therapy was decreased by 5% per week and stopped 6 months after transplantation. Serum samples for the determination of cyclosporine levels were obtained three times weekly, 12 hours after the last cyclosporine dose. Samples were analyzed by radioimmunoassay.

Patients randomized to receive methotrexate alone were given methotrexate, 15 mg/m² IV on day 1 and 10 mg/m² on days 3, 6, 11, and 18 and once weekly thereafter until day 102. The methotrexate dose was reduced or occasionally held in case of renal impairment.

Sixteen patients given methotrexate/cyclosporine and 18 given methotrexate were treated in laminar airflow isolation rooms with skin and gut decontamination assignment to these rooms depended on availability. The remainder were treated in conventional reverse-isolation rooms.

Documentation of hematopoietic engraftment and assessment and grading and treatment of acute and chronic GVHD were done as previously described. Primary therapy of established grades II to IV acute GVHD consisted of methylprednisolone, 2 mg/kg/d, either IV or orally in divided doses for a period of seven to 14 days when the patient's status was reevaluated. Progressive GVHD was treated with antithymocyte globulin or, in the case of methotrexate-treated patients, with cyclosporine. Chronic GVHD was treated with methylprednisolone, either alone or combined with azathioprine. Routine marrow aspirates and cytogenetic studies were usually performed on days 14, 21, 28, 56, 84, and 365 postgrafting to assess the quality of engraftment.

Results of the study were analyzed as of Aug 31, 1985. The primary factor analyzed was the occurrence of grades II to IV acute GVHD. The summary analyses of the trial used Cox's relative risk regression model. Kaplan-Meier estimates of the incidence of acute GVHD and of the survival curves for the two treatment arms were also calculated.

Table 1. Patients Studied

<table>
<thead>
<tr>
<th>No. of patients studied</th>
<th>Methotrexate/Cyclosporine</th>
<th>Methotrexate</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td></td>
<td>24</td>
</tr>
</tbody>
</table>

Table 2 summarizes the postgrafting results.

**Engraftment, transfusion support, early toxicities, and graft rejection.** All 46 patients showed initial evidence of hematopoietic engraftment as determined by rising peripheral blood counts, marrow cellularity, and blood genetic marker studies. The speed of recovery of granulocyte counts and the time to discontinuation of platelet and RBC transfusion support were identical in the two groups, as was the incidence of oral mucositis. There was a significantly higher incidence of elevated bilirubin levels during the first 14 days after grafting in patients given methotrexate/cyclosporine compared with methotrexate alone. Renal function impairment was a frequent complication in patients given methotrexate/cyclosporine. Hypertension (diastolics ≥90 mm Hg on at least two separate daily measurements) was seen in nine patients given methotrexate/cyclosporine and six given methotrexate; four of the six patients receiving methotrexate prophylaxis, however, were being treated with additional cyclosporine for established GVHD. There was no signific-
cant difference between the two groups in the number of days spent in the hospital.

Cyclosporine trough levels for weeks 1, 2, 3, 4, 5, 6, and 7 (means ± 1 SD) were 99 ± 74, 148 ± 116, 134 ± 126, 187 ± 207, 105 ± 103, 120 ± 94, and 130 ± 82 ng/dL, respectively. Patients received approximately 75% of the calculated cyclosporine dose. Renal function impairment or marrow toxicity led to reductions in methotrexate doses in some patients. Specifically, 13 patients given the combination of drugs received 100% of the calculated methotrexate dose, three received between 80% and 90%, and six received less than 80%. Eight of the patients given methotrexate alone received 100% of the calculated drug dose, five received between 80% and 97%, and 11 received less than 80%. A major reason for reductions or omissions of methotrexate doses in this group of patients was severe liver function abnormalities in patients with grades III and IV acute GVHD.

One patient in each group rejected the marrow graft following initial engraftment. Both patients successfully received retransplants 194 and 203 days after the initial transplant, using marrow from the same donor and following a conditioning regimen consisting of cyclophosphamide, 50 mg/kg, on each of four successive days alternating every 12 hours with antithymocyte globulin, 30 mg/kg IV, for a total of three doses. Twenty-one patients given methotrexate alone (Table 2). There was no statistically significant difference between the two patient groups with regard to the probability of chronic GVHD developing (Table 2). Mean

Table 2. Posttransplantation Results (Cont’d)

<table>
<thead>
<tr>
<th>Table 2. Posttransplantation Results</th>
<th>Methotrexate/Cyclosporine</th>
<th>Methotrexate</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median day to discontinuation of RBC transfusion (range)</td>
<td>53.5 (8–119)</td>
<td>79 (6–339)</td>
<td>.88*</td>
</tr>
<tr>
<td>No. patients with marrow graft rejection</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Median days in hospital (range)</td>
<td>40 (27–94)</td>
<td>45 (28–244)</td>
<td>.15*</td>
</tr>
<tr>
<td>Oral mucositis (no. of patients)</td>
<td>None 12</td>
<td>14</td>
<td>NS</td>
</tr>
<tr>
<td>Mild 7</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate 2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe 1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range) peak bilirubin level during first 2 weeks (mg/100 mL)</td>
<td>2.9 (0.8–12.1)</td>
<td>1.2 (0.6–17.7)</td>
<td>.003*</td>
</tr>
<tr>
<td>Doubling of creatinine level greater than baseline or creatinine level greater than 2 mg/100 mL (no. of patients)</td>
<td>13</td>
<td>8</td>
<td>&lt;.10†</td>
</tr>
<tr>
<td>Dialysis (no. of patients)</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Hypertension (no. of patients)</td>
<td>9</td>
<td>6§</td>
<td></td>
</tr>
<tr>
<td>Causes of death (no. of patients)</td>
<td>CMV IP with/without GVHD 0/0</td>
<td>2/0</td>
<td></td>
</tr>
<tr>
<td>Idiopathic IP with/without GVHD</td>
<td>1/0</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>Other infections with/without GVHD</td>
<td>1/1</td>
<td>6/0</td>
<td></td>
</tr>
<tr>
<td>Veno-occlusive disease of liver, hemorrhage</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Acute GVHD (no. of patients)</td>
<td>Grade 0 16</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>0</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Chronic GVHD (no. of patients)</td>
<td>Yes 9</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>10</td>
<td>8</td>
<td>.69#</td>
</tr>
</tbody>
</table>

Abbreviations: NS, not significant; CMV, cytomegalovirus; IP, interstitial pneumonia.

*Rank sum test statistic.
†Chi-square test.
§Defined as diastolics ≥90 mm Hg on at least two separate daily measurements.
#Four of the six patients were being treated with additional cyclosporine for established GVHD.
Excludes patients who rejected their first graft.
In patients who lived at least 6 months after transplantation with sustained first grafts.
Log rank statistics.
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Fig 1. Probability of grades II to IV acute GVHD developing in 46 patients with severe aplastic anemia given marrow grafts from HLA-identical family members and GVHD prophylaxis with either methotrexate/cyclosporine (MTX + CSP) or methotrexate alone (MTX) (Kaplan-Meier product limit estimates). Tick marks represent surviving patients. Survival is as of Aug 31, 1985.

Fig 2. Probability of survival in 46 patients with severe aplastic anemia given marrow grafts from HLA-identical siblings and GVHD prophylaxis with either methotrexate/cyclosporine (MTX + CSP) or methotrexate alone (MTX) (Kaplan-Meier product limit estimates). Tick marks represent surviving patients.

(median) Karnofsky performance scores among patients with chronic GVHD in the two groups are 85% (90%) and 83% (80%), respectively. All nine surviving patients with chronic GVHD in the methotrexate/cyclosporine-treated group and all six in the methotrexate-only group are presently receiving immunosuppressive treatment.

Survival. The projected survival rate at 2 years is 82% for patients receiving methotrexate/cyclosporine compared with 60% for those receiving methotrexate alone (Fig 2). A Cox regression analysis (Table 3), also considering other prognostic factors, showed a suggestive survival advantage for the group receiving methotrexate/cyclosporine ($P = 0.062$). A significant beneficial influence on survival was the treatment of patients in laminar airflow room isolation. The influence of age on survival could not be assessed with this model since no patient below the age of 15 in either treatment group died.

Causes of death. Predominant causes of death were interstitial pneumonias and bacterial/fungal infections associated with GVHD (Table 2). This was most marked among patients receiving methotrexate only, with eight of the nine deaths attributable to these complications.

One patient given methotrexate died of interstitial pneumonia not associated with GVHD. He had had an unsuccessful course of immunosuppressive therapy with antithymocyte globulin before transplantation.

Among the patients given methotrexate/cyclosporine, veno-occlusive disease of the liver developed in one 43-year-old, and this patient died of hemorrhage on day 12. Another 40-year-old patient whose aplastic anemia was due to gold therapy of rheumatoid arthritis had an unsuccessful course of antithymocyte globulin therapy before transplantation. She was admitted with multiple bacterial/fungal brain and liver abscesses and died of these complications three days after transplantation.

Overall, nine of the 14 deaths in the two patient groups were related to complications from acute GVHD. Two additional patients died of problems associated with chronic GVHD.

DISCUSSION

Analogous with results in marrow-transplanted dogs, three prospective clinical trials in patients with leukemia comparing methotrexate and cyclosporine treatment as single agents have shown either no or only marginal decreases in the incidence of acute GVHD in cyclosporine-treated patients without improving survival. Subsequent experimental studies showed an impressive reduction of GVHD in dogs given a combination of methotrexate and cyclosporine. The survival rate of dogs given DLA-nonidentical unrelated marrow grafts was increased from 6% with either drug alone to 35% with the combination, and that of recipients of DLA-haploidentical littermate marrow from 8% to 70%. The current clinical trial was based on these experimental data. It showed methotrexate/cyclosporine therapy to be superior to methotrexate alone in preventing acute GVHD, thereby reducing the frequency of fatal infections and improving survival of patients given marrow grafts for severe aplastic anemia. Adjusting for age, the survival of patients given methotrexate alone was comparable to that reported previously. FDA regulations prohibited the use of cyclosporine in patients below the age of 12 years for most of the current trial; historically, these young patients have a lower incidence of acute GVHD and better survival than older patients. The six patients excluded from this study because of young age were given methotrexate as GVHD prophylaxis, and all are surviving.

The long-term success of marrow grafting for the treat-
PREVENTION OF ACUTE GVHD BY MTX/CSP

The effectiveness of methotrexate/cyclosporine combination therapy for reducing the incidence and severity of acute GVHD has been demonstrated in several randomized prospective studies.3,31-34 The combination has been shown to reduce significantly the overall incidence of GVHD, whereas comparable patients in the methotrexate/cyclosporine-treated group survived long enough to be at risk for chronic GVHD.

The use of laminar airflow room isolation, previously shown to reduce significantly the overall incidence of GVHD and increase survival,40 had a less pronounced effect on the incidence of acute GVHD in the present study while continuing to show a significant beneficial effect on long-term survival. The effectiveness of methotrexate/cyclosporine in reducing the incidence and severity of acute GVHD may have in part obscured the beneficial influence of laminar airflow room isolation.

A major concern with regard to the use of any postgrafting immunosuppression is drug toxicity. The speed of recovery of blood counts and the incidence and severity of oral mucositis were similar in the two groups of patients; however, patients in the methotrexate/cyclosporine-treated group showed a higher incidence of transient liver function abnormalities, predominantly bilirubin elevations during the first 14 days. Presumably this was the result of an additive or synergistic toxic effect of methotrexate/cyclosporine combined with the toxicity of the conditioning regimen. Indeed, one patient in the methotrexate/cyclosporine group, aged 43 years, died of complications associated with severe veno-occlusive disease of the liver, presumably the result of drug toxicity. Other previously reported cyclosporine toxicities such as nephrotoxicity41 and hypertension42 were frequent findings in the present study. Nephrotoxicity is a particular problem during the early postgrafting period, potentiated by the use of nephrotoxic antibiotics including amphotericin B.43 The early renal function abnormalities required reduction or omission of methotrexate and cyclosporine doses in some patients. The impact of drug dose reductions or omissions on subsequent development of acute GVHD is difficult to evaluate. The fact that more patients given methotrexate required dialysis was probably related to the fact that these patients had severe acute GVHD for which they were given additional treatment with cyclosporine.

We conclude that the combination of methotrexate and cyclosporine resulted in a significant decrease in the incidence and severity of acute GVHD in patients who received transplants for severe aplastic anemia, with a resultant improvement in survival. However, the latter conclusion is only tentative since follow-up of these patients is still short, with the longest survivor at 4 years.

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Marrow transplantation for severe aplastic anemia: methotrexate alone compared with a combination of methotrexate and cyclosporine for prevention of acute graft-versus-host disease

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