Therapy of Severe Aplastic Anemia in Young Adults and Children With Allogeneic Bone Marrow Transplantation

By P.B. McGlave, R. Haake, W. Miller, T. Kim, J. Kersey, and N.K.C. Ramsay

During an 8-year period, 28 young adults (median age 27 years) and 30 children (median age 10 years) with severe aplastic anemia have received allogeneic bone marrow transplantation (BMT) from major histocompatibility locus-matched sibling donors after preparation with cyclophosphamide and total lymphoid irradiation (TLI). All recipients were previously transfused. Comparison of post-bone marrow transplantation events in adults and children reveals equivalent median time to engraftment, median duration of hospitalization, median Karnofsky assessment of activity, and equivalent low rejection rate. Although the incidence of moderate and severe acute graft-versus-host disease (GVHD) and of extensive chronic GVHD was greater in adults than in children, the projected survival at 4 years of adults (67%)

Transplantation of bone marrow from HLA-identical siblings has been used successfully to treat severe aplastic anemia. Rejection rates of <10% are common in untransfused recipients. Early reports suggested that blood transfusion prior to transplantation resulted in a significant incidence of graft failure and an adverse effect on long-term survival. Modifications of the preparative regimens have decreased the rejection rate in this transfused population. Improvements in transfusion practices may also have contributed to the reduction in incidence of rejection.

Total lymphoid irradiation (TLI) is a potent form of immunosuppression both in animal models and in humans. We have used the combination of TLI and high-dose cyclophosphamide to prepare previously transfused aplastic anemia patients for allogeneic BMT since 1977. In this report, we update our experience with this approach and compare results in children and in young adults.

Patients and Methods

Since November 1977, 28 young adults (median age 27 years, range 18 to 45 years) and 30 children (median age 10 years, range 2 to 17.6 years) received BMT as therapy for severe aplastic anemia. Pretransplant recipient and donor characteristics are presented in Table 1. All patients met established criteria for the diagnosis of severe aplastic anemia. All patients were transfused, and all but one had received more than five transfusions, ie, had been exposed to products from more than five separate blood donors prior to BMT. In addition to significant differences in donor and recipient age, adults and children differed in previous transfusion history (<20 v >20 transfusions) and in marrow cell dose. All patients or their legal guardians signed informed consent approved by the Committee on the Use of Human Subjects in Research at the University of Minnesota.

Patients were prepared for transplantation with a regimen designed to minimize rejection including cyclophosphamide, 50 mg/kg/day intravenously (IV) (day -6 to day -3) and TLI, 750 cGy midline dose at the umbilicus (day -1). TLI was given in a single dose at a rate of 26 cGy/min with a 4-MV linear accelerator. Patients then received marrow from major histocompatibility (MHC)-matched sibling donors (day 0). All patients received graft-versus-host disease (GVHD) prophylaxis with methotrexate. Twenty-nine patients received additional GVHD prophylaxis with equine antithymocyte globulin (ATG) and prednisone. Four patients received 5 mg/day of OKT3 given IV from day -8 to day +20 in addition to methotrexate and prednisone. Acute GVHD organ involvement was graded I through IV. Chronic GVHD was scored as limited or extensive. The term "advanced GVHD" was applied to patients who developed either grade II or greater acute GVHD or extensive chronic GVHD. Mild cutaneous acute GVHD and limited chronic GVHD were treated with topical steroid cream. Advanced GVHD was treated with regimens containing systemic steroids and, in some cases, adjunctive ATG, cyclosporine, or azathioprine therapy.

Statistical methods. Clinical and laboratory data were retrieved from the University of Minnesota Bone Marrow Transplant Database, which contains systematically and prospectively collected data for all BMT recipients and donors. Univariate analyses were performed using the Kaplan-Meier product-limit method to assess the effects of various factors on survival. The Mantel-Cox statistic was used to compare the difference in survival between groups. The sole exception involved assessment of the possible influence of GVHD on survival. Because GVHD is not a baseline patient characteristic and occurs some time after transplant, it was treated as a time-dependent covariate in a Cox proportional-hazards regression analysis. Factors that were significantly associated with survival in univariate analysis were then entered into a Cox stepwise multivariate regression analysis to determine their independent contributions to survival.

Results

Projected survival at 4 years for the adult group is 67% (95% CI 49% to 85%), whereas that of the children is 73%.
Projected long-term survival of 30 children (<18 years) and 28 adults (18 to 44 years) undergoing BMT for SAA. One surviving child who experienced marrow failure and was retransplanted is a transfusion-free survivor at +78 months.

**Table 1. Pretransplant Donor and Recipient Characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Adults</th>
<th>Children</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>28</td>
<td>30</td>
<td>NS</td>
</tr>
<tr>
<td>Interval from diagnosis to BMT</td>
<td>47 days</td>
<td>44 days</td>
<td>NS</td>
</tr>
<tr>
<td>Transfusion history</td>
<td>&lt;20 transfusions</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>Recipient age</td>
<td>27 yr</td>
<td>10 yr</td>
<td><em>P</em> = .0001</td>
</tr>
<tr>
<td>Donor age</td>
<td>25 yr</td>
<td>10 yr</td>
<td><em>P</em> = .0001</td>
</tr>
<tr>
<td>Recipient sex</td>
<td>M</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Donor sex</td>
<td>M</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Marrow cell dose</td>
<td>× 10^5/kg of recipient body weight</td>
<td>3.2</td>
<td>3.8</td>
</tr>
</tbody>
</table>

(95% CI 57% to 89%) (*P* = .58) (Fig 1). The latest death in the series occurred at 11 months following BMT. All survivors are transfusion independent. Characteristics of post-BMT of the two groups (Table 2) including median times to engraftment, median durations of hospitalization, median Karnofsky assessments of activity in survivors, and rejection rates are equivalent.

**GVHD.** The incidence and severity of GVHD in adults and in children is shown in Table 2. Kaplan-Meier analysis revealed a higher incidence of moderate and severe acute GVHD at 100 days in adults (54%; 95% CI 35% to 73%) than in children (24%; 95% CI 9% to 40%) (*P* = .08), and a significantly higher long-term projected incidence of extensive chronic GVHD in adults (56%; 95% CI 35% to 78%) than in children (12%; 95% CI 0% to 25%) (*P* = .008). The long-term projected incidence of advanced GVHD in adults of 68% (95% CI 49% to 86%) was significantly greater than the 36% projected incidence (95% CI 18% to 54%) seen in children (*P* = .02) (Fig 2). Twelve patients in this series have required systemic steroid therapy and, in some cases, adjunctive therapy with azathioprine for extensive chronic GVHD. Three of these 12 patients have died, whereas 8 patients have experienced complete resolution of chronic GVHD and are no longer receiving immunosuppressive therapy. One patient is receiving low doses of azathioprine as therapy for persistent cutaneous chronic GVHD.
**Graft failure.** Three of 58 patients experienced non-engraftment or rejection following BMT. A 9-year-old girl (UPN 96) with idiopathic severe aplastic anemia who had received <20 transfusions prior to transplantation failed to engraft following an infusion of \(3.9 \times 10^8\) nucleated bone marrow cells/kg of recipient weight from her MHC-matched brother. A second infusion of \(4.4 \times 10^8\) cells per kilogram of recipient weight from the same donor following preparation with cyclophosphamide and TLI, and ATG was unsuccessful, as was a third infusion of \(1.7 \times 10^8\) cells/kg of recipient weight. The patient died at +99 days with *Escherichia coli* sepsis and *Aspergillus sp* pneumonia. No evidence of engraftment could be found at time of death.

A 7-year-old girl (UPN 103) with idiopathic severe aplastic anemia who had received <20 transfusions prior to transplantation had successful engraftment at day +25 after receiving \(3.9 \times 10^8\) cells/kg of recipient weight from her MHC-matched sister. Twenty-nine months later, the patient returned with recurrent severe aplastic anemia. After preparation with cyclophosphamide, 50 mg/kg/day (day -5 to day -2) and TBI 300 cGy (day -1), the patient received \(3 \times 10^8\) bone marrow cells/kg of recipient weight from the same donor and engrafted at day +19. She is now alive and well at +45 months following a second BMT.

A 26-year-old man (UPN 410) with idiopathic severe aplastic anemia who had received >20 transfusions prior to BMT as well as an unsuccessful course of equine ATG (Minnesota) failed to establish a graft following preparation with cyclophosphamide and TLI, and an infusion of \(3.2 \times 10^8\) cells/kg of recipient weight donated by his MHC-matched brother. Peripheral blood evidence of engraftment occurred on day +19 and was confirmed by bone marrow biopsy. Aplasia recurred at day +45 and was treated by infusion of \(3.1 \times 10^8\) cells/kg of recipient weight using bone marrow cells from the same donor following preparation with cyclophosphamide, cytosine arabinoside, and TBI. Engraftment did not occur, and the patient died at day +120 with *Aspergillus sp* pneumonia.

**Thyroid dysfunction.** "Compensated hypothyroidism," defined as elevation of serum thyroid stimulating hormone (TSH) serum levels >10 μIU/ml on at least one occasion or between 7 to 9.9 μIU/ml on at least two occasions in the presence of a normal T4 index, occurred in 13 of 37 recipients studied at >2 years after BMT. "Primary hypothyroidism," defined as concomitant depression of the serum T4 index (<5) and the above-defined serum TSH elevation, occurred in an additional three recipients.

**Pneumonia.** In this series, pneumonia occurred in 31 of 58 patients. Etiologies included cytomegalovirus (CMV) (5 cases), fungus (3 cases), bacterial infection (3 cases), *Pneumocystis carinii* (3 cases), acute respiratory distress syndrome (ARDS) (1 case), and pulmonary hemorrhage (1 case). In 15 cases, no etiology was determined.

**Cause of death.** Seventeen of the 58 patients studied have died. Primary causes of death are listed in Table 3. In 9 of the 17 cases, pneumonia was considered the principal clinical event responsible for death. In one case (UPN 129) rapid onset of fatal pulmonary edema occurred in a patient receiving concomitant amphotericin B empiric therapy and WBC infusion. In a second case (UPN 525), death was related to uncontrollable hemorrhage into the lung after ear, nose, and throat (ENT) surgery. In the remaining 7 cases fatal pneumonia could be attributed to CMV (5 cases), *Candida sp* (1 case), or a bacterial pathogen (1 case). In 4 cases, sepsis with either bacterial pathogens (3 cases) or with CMV (1 case) accounted for death. Meningitis from *Staphylococcus sp* and from *E coli*, respectively, occurring in patients with advanced GVHD, accounted for two additional deaths related to infection. Finally, two patients previously described (UPN 96, UPN 410) died of infectious complications associated with graft failure and prolonged pancytopenia. Advanced GVHD was considered a contributing feature in 11 of the 17 deaths.

**Survival.** Univariate Kaplan-Meier analyses were performed to assess the influence of patient characteristics on survival. No significant correlation with survival could be found when recipient age or sex, donor age or sex, recipient/donor sex match, or GVHD prophylaxis were considered. Prolonged interval from diagnosis to BMT defined as greater than the median interval of 46 days \((P = .01)\) and an extensive pretransplant transfusion history (>20 U) \((P = .02)\) predicted decreased survival. The occurrence of advanced GVHD was treated as a time-dependent covariate in a Cox regression analysis of survival. Those with advanced GVHD had significantly decreased survival \((P = .0004)\). Survival was also analyzed by decade of recipient age (Table 4). Although patients aged >30 years had a trend toward poorer survival, there was no significant difference in survival among the four age groups \((P = .75)\).

A Cox multivariate analysis was then conducted to assess the independent contribution of factors that were significant receiving concomitant amphotericin B empiric therapy and WBC infusion. In a second case (UPN 525), death was related to uncontrollable hemorrhage into the lung after ear, nose, and throat (ENT) surgery. In the remaining 7 cases fatal pneumonia could be attributed to CMV (5 cases), *Candida sp* (1 case), or a bacterial pathogen (1 case). In 4 cases, sepsis with either bacterial pathogens (3 cases) or with CMV (1 case) accounted for death. Meningitis from *Staphylococcus sp* and from *E coli*, respectively, occurring in patients with advanced GVHD, accounted for two additional deaths related to infection. Finally, two patients previously described (UPN 96, UPN 410) died of infectious complications associated with graft failure and prolonged pancytopenia. Advanced GVHD was considered a contributing feature in 11 of the 17 deaths.

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A Cox multivariate analysis was then conducted to assess the independent contribution of factors that were significant

**Table 3. Cause of Death**

<table>
<thead>
<tr>
<th>Principal Cause*</th>
<th>(GVHD)†</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonia</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>CMV</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td><em>Candida sp</em></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ARDS</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sepsis</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Meningitis</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Graft failure</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>17</td>
</tr>
</tbody>
</table>

*Single clinical feature considered most important in patient death. †GVHD or GVHD therapy considered a contributing factor in patient death.

**Table 4. Projected Long-term Survival by Decade**

<table>
<thead>
<tr>
<th>Age</th>
<th>Number</th>
<th>Survival (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-9</td>
<td>16</td>
<td>75</td>
<td>54-96</td>
</tr>
<tr>
<td>10-19</td>
<td>20</td>
<td>75</td>
<td>55-94</td>
</tr>
<tr>
<td>20-29</td>
<td>12</td>
<td>67</td>
<td>40-93</td>
</tr>
<tr>
<td>30-44</td>
<td>10</td>
<td>58</td>
<td>27-90</td>
</tr>
</tbody>
</table>

Table 4 depicts the projected long-term survival of BMT recipients analyzed by age. There is no significant difference in projected survival among the four groups \((P = .75)\)
In univariate analyses. Because the effect of recipient age on outcome was of interest, it was included in the analysis and was forced into the model at the first step. The results of this analysis are presented in Table 5. Advanced GVHD \((P = .005)\) and a prolonged interval from diagnosis to transplant \((P = .01)\) predicted poorer outcome. Neither recipient age nor previous transfusion history significantly influenced survival when occurrence of advanced GVHD and interval from diagnosis to BMT were taken into account.

**DISCUSSION**

In this report, we demonstrate that transplantation of bone marrow taken from MHC-matched sibling donors provides excellent survival for previously trans fused children and young adults with severe aplastic anemia. Preparation of recipients with high-dose cyclophosphamide and TLI is associated with a low rejection rate \((P = .005)\). Early reports suggested that transplantation of previously transfused patients resulted in a significant incidence of graft failure and an adverse effect on long-term survival.\(^{14}\) Graft failure was attributed to nonspecific sensitization of the recipient by antigens found in transfused blood products.\(^{21,22}\)

Subsequently, modifications of the preparative regimen have been designed to reduce the rate of graft failure in transfused recipients. Addition of nonirradiated donor peripheral blooduffy coat following marrow infusion has reduced the incidence of graft failure from 32% to 14%.\(^{3}\) Infusion of buffy coat, however, is associated with an increased incidence of chronic GVHD.\(^{23}\)

Immunosuppression with a combination of total body irradiation \((300 \text{ cGy})\) given in a single dose and high-dose cyclophosphamide resulted in a projected survival rate of 61% and rejection in only 1 of 44 previously transfused severe aplastic anemia patients. A high incidence of interstitial pneumonitis and a high mortality in adults, however, was reported.\(^{6}\) Addition of cyclosporine to the BMT preparative regimen has been reported to reduce the risk of early graft failure.\(^{7}\) A subsequent report from the same authors, however, demonstrated that unexpected late graft failures may occur after withdrawal of cyclosporine therapy.\(^{24}\) When immunosuppression with cyclosporine is combined with high-dose cyclophosphamide, both early graft failure and fatal venoocclusive disease of the liver can occur.\(^{25}\) Preparative immunosuppression with a combination of procarbazine, rabbit antithymocyte serum \(\text{(ATS)}\), and high-dose cyclophosphamide has also been reported to provide a low rejection rate \((10\%)\) and acceptable projected long-term survival \((61\%)\) in a group of relatively young recipients \(\text{(median age 17 years)}\).\(^{8}\)

The 5% rejection rate in this series compares favorably with rejection rates reported after BMT therapy of either untransfused or previously transfused aplastic anemia patients. This low rejection rate is probably the result of preparative immunosuppression with TLI, which was first shown to be immunosuppressive in animal models.\(^{10,11}\) Other researchers have reported preliminary results suggesting a low rejection rate after preparation of transfused aplastic patients with low-dose TLI.\(^{26}\) Radiation ports used to deliver TLI include portions of the thyroid gland. In this series, 16 of 37 recipients studied at \(>2\) years following transplantation had chemical evidence of hypothyroidism.\(^{27,28}\) Endocrine abnormalities occurring after preparation with single-dose TBI and allogeneic BMT have been reported.\(^{29}\) Hypothyroidism occurring after mantle irradiation therapy of Hodgkin’s disease is also common, often clinically insignificant, and sometimes reversible without therapy.\(^{30,31}\) TLI may have similar adverse effects when used for bone marrow transplantation therapy of severe aplastic anemia.

Pneumonia was a principal clinical feature in 9 of the 17 deaths in this series. The contribution of irradiation to the development of fatal pneumonia is difficult to determine. Damage to the pulmonary bed itself is unlikely since individually tailored lung shielding permitted <10% of the total calculated dose of irradiation to penetrate the center of the lung block in all recipients. In seven of the nine cases of fatal pneumonia, an infectious pathogen was demonstrated. The additive immunosuppressive effect of TLI and cyclophosphamide may have predisposed recipients to pneumonia in these cases. Most recipients with fatal pneumonia, however, also were receiving treatment for advanced GVHD. GVHD and therapy for GVHD have been associated with the development of pneumonia following allogeneic BMT for leukemia.\(^{32}\) The relative importance of these etiologic features on the development of fatal infectious pneumonia is not known.

The long-term, projected incidence of advanced GVHD was 67% in adults and 36% in children, and advanced GVHD contributed significantly to mortality in this series. The reason for the disparity between the projected incidence of GVHD in adults and in children is not obvious. An increased incidence of GVHD has occurred in severe aplastic anemia patients receiving nonirradiated donor buffy coat infusions.\(^{23}\) Adults in this series, however, received a lower median cell dose per kilogram of recipient weight \((3.2 \times 10^8)\) than did children \((3.8 \times 10^8)\) \((P = .05)\), and neither children nor adults received donor buffy coat infusions. The relative

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Relative Risk</th>
<th>Significance (p) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advanced GVHD</td>
<td>5.9</td>
<td>.002</td>
</tr>
<tr>
<td>Prolonged interval from diagnosis to BMT</td>
<td>2.4</td>
<td>.01</td>
</tr>
<tr>
<td>History of &gt;20 transfusions prior to BMT</td>
<td>—</td>
<td>.10</td>
</tr>
<tr>
<td>Age &gt;18 years</td>
<td>—</td>
<td>.58</td>
</tr>
</tbody>
</table>

Table 5 depicts results of stepwise multivariate analysis correlating various risk features with death in 58 transfused patients undergoing BMT for SAA.
importance of older donor age in the development of GVHD is difficult to assess in this series since donor age parallels recipient age in both the older and in the younger group. Despite the increased incidence of advanced GVHD in adults, no significant disparity in parameters associated with outcome (including time to engraftment, rejection rate, median length of hospitalization, or median Karnofsky assessment of survivors) could be found. Recent reports suggest that prophylaxis with the combination of methotrexate and cyclosporine after allogeneic BMT therapy for severe aplastic anemia can result in an extremely low cumulative incidence of moderate or severe acute GVHD (14%) with a low rejection rate and with excellent projected survival. Studies that compare the relative merits of our current acute GVHD prophylaxis to prophylaxis with the combination of methotrexate, cyclosporine, and prednisone are underway at our institution.

In this study, excellent long-term transfusion-free survival occurred in both young adults (18 to 44 years) and in children (1 to 17 years). Comparison of survival by decade demonstrated no significant difference, although only 10 adult recipients were between the ages of 30 and 44 years. An inverse correlation of survival and increasing recipient age following BMT for severe aplastic anemia has been reported; however, the comparable survival in children and young adults in our series is in agreement with two recently reported analyses.

The observation has been made that patients receiving allogeneic BMT therapy for severe aplastic anemia in the “modern era” have lower rejection rates and better survival than patients transplanted with similar preparative regimens in earlier years. Low rejection rates, comparable to those that are observed after transplant of untransfused severe aplastic anemia patients, have been attributed to a decreased incidence of recipient sensitization accompanying improvements in blood product transfusion procedures. Better overall survival may be attributable to expedited therapy with BMT, and better pretransplant and posttransplant support. The results of our study are in agreement with reports of better survival in recently transplanted patients. In our experience, projected long-term transfusion-free survival of 28 patients (median age 14 years) transplanted from 1977 to 1981 is 61% (95% CI 43% to 79%), whereas projected long-term survival of 30 patients transplanted from 1982 to 1986 (median age 21 years) is 79% (95% CI 64% to 94%) (P = .10).

Allogeneic bone marrow transplantation therapy for severe aplastic anemia after preparation with a combination of cyclophosphamide and TLI provides excellent transfusion-free survival and an extremely low rate of graft failure in young adults and in children. Results of this approach compare favorably with those that have been observed after transplantation with other reported preparative regimens or after therapy with alternative forms of immunosuppression.

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