BONE MARROW TRANSPLANTATION IN 107 PATIENTS WITH SEVERE APLASTIC ANEMIA USING CYCLOPHOSPHAMIDE AND THORACO-ABDOMINAL IRRADIATION FOR CONDITIONING: LONG-TERM FOLLOW-UP

By E. Gluckman, G. Socie, A. Devergie, H. Bourdeau-Esperou, R. Traineau, and J.M. Cosset

Since 1980, 107 consecutive patients (pts) underwent bone marrow transplantation (BMT) for nonconstitutional severe aplastic anemia (SAA) at our institution. All received conditioning with Cytoxan (150 mg/kg) and thoraco-abdominal irradiation (6 Gy) from an HLA-identical sibling donor. Mean age was 19 years (5 to 46 years). Forty-nine pts had less than 0.2 x 10^9/L PMN and 53 failed to respond to previous immunosuppressive therapy before BMT. Graft-versus-host disease (GVHD) prophylaxis consisted of methotrexate (22 pts), cyclosporine (52 pts), or both (33 pts). With a median follow-up of 45 months (12 to 120 months), overall actuarial survival was 68% (confidence interval 95%: 97%). Of 16 factors tested, five were shown to adversely influence survival by multivariate analysis: grade ≥ 2 acute GVHD (relative risk [RR]: 5.5), prior immunosuppressive therapy (RR: 3.5), female as donor (RR: 2.4), nonidiopathic SAA (RR: 2), and more than 0.2 x 10^9/L PMN AA (RR: 2). Because acute GVHD was the most potent factor for survival, we analysed risk factors for acute GVHD. By multivariate analysis, 2 of 14 factors tested were independent: male as recipient (RR: 3) and previous alloimmunization of the donor (RR: 4.3). On long-term follow-up, chronic GVHD was observed in 49 pts of 89 surviving more than 100 days (55%). Multivariate analysis showed that infection before transplant (RR: 1.3) and previous history of acute GVHD (RR: 1.8) were associated with an increased risk of chronic GVHD.

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Table 1. Patient Pretransplant Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etiology</td>
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<td></td>
</tr>
<tr>
<td>Idiopathic</td>
<td>82/107</td>
<td>77</td>
</tr>
<tr>
<td>Toxic</td>
<td>2/107</td>
<td>1</td>
</tr>
<tr>
<td>Posthepatitis</td>
<td>17/107</td>
<td>16</td>
</tr>
<tr>
<td>PNH</td>
<td>6/107</td>
<td>6</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>66/107</td>
<td>62</td>
</tr>
<tr>
<td>Female</td>
<td>41/107</td>
<td>38</td>
</tr>
<tr>
<td>Infection 1 wk before transplant</td>
<td>40/108</td>
<td>37</td>
</tr>
<tr>
<td>Immunization</td>
<td>45/108</td>
<td>42</td>
</tr>
<tr>
<td>Previous treatments</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Androgens</td>
<td>41/108</td>
<td>38</td>
</tr>
<tr>
<td>ATG</td>
<td>25/108</td>
<td>23</td>
</tr>
<tr>
<td>CSA</td>
<td>5/108</td>
<td>5</td>
</tr>
<tr>
<td>Associations</td>
<td>23/108</td>
<td>21</td>
</tr>
<tr>
<td>Interval between diagnosis and transplant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1 mo</td>
<td>4/108</td>
<td>4</td>
</tr>
<tr>
<td>1-2 mo</td>
<td>26/108</td>
<td>24</td>
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<tr>
<td>2-5 mo</td>
<td>33/108</td>
<td>31</td>
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<tr>
<td>&gt;5 mo</td>
<td>45/108</td>
<td>42</td>
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<tr>
<td>Donor-recipient sex</td>
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<td></td>
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<tr>
<td>M → M</td>
<td>38/108</td>
<td>35</td>
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<tr>
<td>M → F</td>
<td>29/108</td>
<td>27</td>
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<tr>
<td>F → M</td>
<td>24/108</td>
<td>22</td>
</tr>
<tr>
<td>F → F</td>
<td>17/108</td>
<td>16</td>
</tr>
</tbody>
</table>

Abbreviation: PNH, paroxystic nocturnal hemoglobinuria.

median blood counts were: leukocytes $2.5 \times 10^9$/L (range 0.5 to 10), reticulocytes $14.5 \times 10^9$/L (range 0 to 130), granulocytes 16% (range 0% to 79%), platelets $17.5 \times 10^9$/L (range 0 to 80). Forty-five percent of the patients had less than $0.2 \times 10^9$/L granulocytes before BMT. Donors were HLA-identical, mixed lymphocyte culture (MLC) nonreactive siblings. The median donor age was 19 years (range 1.5 to 52 years).

The patients received a mean bone marrow cell number of $3.4 \times 10^9$/kg (range 1 to 8.5).

For GVHD prophylaxis, 22 patients received MTX alone (20%), 52 CsA (48%), and 33 the combination of MTX and CsA (31%) at dosages previously published.

Statistical analysis was performed on a personal IBM computer with BMDP statistical software. Probability estimates were obtained with the Kaplan-Meier method and differences between survival patterns were evaluated by log-rank statistics. Multivariate proportional hazards regression analyses used the Cox method. Covariates were added to the model in a stepwise fashion. All covariates that added information to the model at the 0.05 significance level as measured by the maximum likelihood ratio test were included. All significance levels were two-sided and all the variables were tested for time dependence.

All patients were fully informed of the details of the procedure according to our Institutional Ethical Committee rules.

Several pretransplant and posttransplant variables were studied for factors affecting survival, AGVHD, and CGVHD. They were: (1) pretransplant variables: age, sex, etiology, blood counts, infection, previous treatment, number of transfusions, immunization against random platelet transfusions, ABO type, and interval between diagnosis and transplant; (2) donor variables: age, sex, immunization, cytomegalovirus (CMV) serology, and ABO type; and (3) posttransplant variables: number of cells transplanted, GVHD prophylaxis, incidence and severity of AGVHD and CGVHD, and infectious complications.

RESULTS

Survival. The actuarial 5-year survival curve shows a 68% long-term survival with a median follow-up time of 45 months (Fig 1). Two patients rejected the transplant, one died with an absence of take, and one patient rejected the transplant after 6 months. He was successfully transplanted for a second time with the same conditioning and a different HLA-identical sibling donor. All other patients had a prompt hematologic reconstitution with complete donor engraftment when it was analyzed by Southern blot analysis using either MS or Y chromosome-specific DNA probes.

In the early posttransplant period, several complications were observed: hemorrhagic cystitis (three cases), liver veno-occlusive disease (one case), interstitial pneumonitis (19 cases), sepsis (23 episodes), aspergillosis (four cases), and Candida sepsis (seven cases). Interstitial pneumonitis (IP) was due to CMV in seven cases, Pneumocystis carinii in three cases, bacteria in four cases, and fungi in two cases. Twelve of 19 patients survived IP. A total of 35 patients died, of whom 14 died after 6 months, infection was directly or indirectly the main cause of death in 26 cases (75%). It was associated, with AGVHD in 13 cases and with CGVHD in nine cases.

In univariate analysis, GVHD had a major impact on actuarial survival, which was 81% in patients with a grade 0 or 1 and 40% in patients with a grade ≥ 2 ($P < .00001$). The other factors affecting survival were: (1) the sex of the donor: the survival was 73% with male donors and 52% with female donors ($P < .02$); and (2) blood counts at the time of transplant: patients with less than $0.2 \times 10^9$/L granulocytes had a survival of 80%, those with more than $0.2 \times 10^9$/L had a survival of 51% ($P = .001$). Two other factors were found statistically significant in the univariate analysis: previous therapy for SAA and etiology. Patients who had received previous treatment with immunosuppression with or without androgens had a 50% survival, whereas patients who had not received treatment with ATG had a survival of 76% ($P = .01$). The etiology was also a prognos-
tic factor: patients with nonidiopathic aplastic anemia had 38% survival compared with 69% in the other group (P = .05). The multivariate analysis is shown in Table 2.

The main factors affecting survival included: (1) pretransplant factors: etiology, severity of aplasia, previous immunosuppression, and donor sex; and (2) a posttransplant factor: the occurrence of AGVHD. Age, methods of prevention of GVHD, number of transfusions before transplant, refractoriness to random donor platelet transfusion before transplant, infection, interval between diagnosis and transplant, and sex match between donor and recipient did not affect survival.

Factors associated with AGVHD. GVHD was the main complication after BMT. It was graded 0 in 30 patients (28%), grade I in 33 patients (30.6%), grade II in 22 patients (20.3%), grade III in 11 patients (11.5%), and grade IV in four patients (3.7%). It was treated with 2 to 5 mg/kg prednisone and, in case of failure, with antithymocyte globulin in 15 cases or anti-T monoclonal antibodies in 13 cases, with a good clinical response in 50% of the cases.

In a univariate analysis of factors associated with AGVHD, the following factors were not found to be associated with an increased incidence of AGVHD: recipient immunization by transfusions, etiology of aplasia, number of transfusions, severity of aplasia, interval between diagnosis and transplant, infection before BMT, ABO and sex mismatch between donor and recipient, positive CMV serology of donor or recipient, and immunosuppressive treatment before BMT. The method of prevention of GVHD did not affect the incidence of GVHD ≥ II: it was 27.3% in the MTX group, 50.3% in the CsA group, and 40% in the CsA and MTX group. Two factors correlated with the occurrence of a grade ≥ II GVHD in a univariate and multivariate analysis (Table 2). The risk increased when the recipient was male (P = .001) and when the donor was immunized by previous transfusions or pregnancies (P = .02).

Factors associated with CGVHD. Eighty-nine patients survived for more than 90 days with engraftment and were at risk of developing CGVHD. Forty had no sign of CGVHD (45%), the mean time of onset of CGVHD was 168 days (range 100 to 220 days), and it was limited in 18 cases and extensive in 31 cases. CGVHD appeared de novo in six cases, after a grade I GVHD in 11 cases, a grade II in 10 cases, and grade III or IV in six cases. It was treated with a combination of prednisone, azathioprine, and CsA. Twelve patients had a moderate response and 35 had a complete or good response to the treatment. In cases of severe chronic extensive GVHD, three patients received thalidomide without effect and six a 1-Gy TAI with a good improvement in five cases. Fourteen patients died after 6 months, and in nine cases death was related to infection associated with severe CGVHD. One patient had fulminant hepatitis B due to a reactivation of a pretransplant infection, two patients died of human immunodeficiency virus (HIV) infection, and two died of infection with late marrow failure. In the univariate and multivariate analyses (Table 2), the risk of CGVHD increased with the presence of infection before BMT and a previous history of AGVHD.

On long-term follow-up, most patients returned to a normal life with normal activities, and signs of CGVHD tended to diminish with time. Four patients were infected with HIV before BMT, two are still alive with a follow-up of 10 and 3 years, and one patient had chronic autoimmune thrombocytopenia. Five patients had osteonecrosis of the femoral head that necessitated surgical hip replacement, and one patient had encephalitis of unknown origin and was lost to follow-up. Five patients had secondary malignant tumours: three epidermoid carcinoma of the mouth mucosa and one a parotid mucoepidermoid carcinoma. One patient had acute lymphoblastic leukemia on recipient's cells 18 months after BMT for SAA. After induction of a complete remission with chemotherapy, he was successfully transplanted a second time with the same donor.

DISCUSSION

Our results show that a conditioning regimen associating CYT and 6-Gy TAI gives a 5-year actuarial survival of 68% in a large series of 107 unselected patients with SAA transplanted in a single center. These results are comparable with other published regimens not using irradiation for conditioning.17,20,23,25

In other series, several pretransplant factors were shown to adversely affect survival; they were increasing age, number of transfusions, refractoriness to random platelet transfusions, and delay between diagnosis and BMT.13,19 None of these factors influenced survival in our series, but the most significant factor was the use of ATG with or without androgens as first-line therapy of SAA. One of the possible explanations of this adverse effect might be that this treatment delays BMT and increases the risk of infection and transfusion immunization. Consequently, BMT with an HLA-identical sibling should be preferred as first-line therapy in patients with SAA. Nonidiopathic aplastic anemia had a decreased survival. Most of them, 17 of 25, were posthepatitis aplastic anemia; this group of patient has usually a more acute onset with very low blood counts, and consequently they had more infection before transplant than the other cases.
Other factors associated with the outcome of BMT were related to donor-recipient sex differences. The use of female donors decreased survival. AGVHD was increased when the donor was immunized by previous pregnancies and when the recipient was male. This has been described previously and confirms the importance of the donor immune status and the role of minor histocompatibility differences between donor and recipient.

Rejection was not a major problem as only two patients rejected the transplant, and one is surviving after a second transplant. This decreased rate of rejection is probably related to the use of irradiation for the conditioning, but the same trend toward the decrease of rejection has also been observed in series without irradiation either with buffy coat cell transfusions of the donor after BMT, or more likely because of the use of CsA alone or associated with MTX for prophylaxis of GVHD. Our patients had an early and prolonged complete chimerism with an absence of detectable host residual cells, while mixed chimerism is a frequent finding in regimens using CYT alone. Late bone marrow failure responding to CsA has been described, leading to the prolongation of CsA therapy after BMT. Mixed chimerism is a poor prognostic factor after transplant for leukemia because of the risk of leukemic relapse. In SAA, the presence of autologous hematopoiesis might lead to the development of myelodysplasia, as observed late after immunosuppressive treatment for SAA, but this has not been described so far.

Interstitial pneumonitis and veno-occlusive disease of the liver were not more frequent or more severe than in other series without irradiation, and this was probably due to our technique of shielding the lungs and part of the right liver lobe.

GVHD was the major factor affecting survival and quality of life, with an incidence of 35.5% of GVHD ≤ II and 55% of chronic GVHD. The incidence of AGVHD was higher than in series not using irradiation. CGVHD had the same incidence in series using donor buffy coat cell transfusions after BMT or the association of CsA and MTX. It was lower in series using CYT alone, perhaps because of the mixed chimerism observed in these series. Factors associated with CGVHD were infection before BMT (P = 0.02) and previous history of AGVHD ≥ II (P = 0.04). The mortality after 6 months due to CGVHD or infection was relatively low (15%), but prolonged immunosuppression, disability, and long-term sequelae of chronic skin and mucosal lesions significantly affected the quality of life in 20% of the patients with CGVHD.

Secondary tumors are the most serious side effect of irradiation. Three patients developed carcinoma of the lip or mouth mucosa at the site of CGVHD lichenoid lesions, and one patient had a malignant tumor of the parotid. One patient developed common pre-B acute lymphoblastic leukemia on host cells 18 months after a complete engraftment with donor cells. A careful review of pretransplant bone marrow smears and biopsy failed to show any evidence of leukemia.

In conclusion, despite the similarity of our results in terms of long-term survival with other series reported in the literature, the high incidence of CGVHD and late malignancies leads to the conclusion that irradiation should not be recommended for preparation of patients with SAA transplanted with an HLA-matched sibling.

Highly immunized patients or patients receiving a transplant from a mismatched related donor, a matched unrelated donor, or a T-cell-depleted marrow have a high risk of rejection and may need an intensification of the conditioning regime.

CGVHD remains a major problem and requires improvement of the methods of long-term immunosuppressive treatment.

Considering the rarity of the disease, these questions could be addressed only by multicenter prospective studies.

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BONE MARROW TRANSPLANT IN SAA LONG-TERM


Bone marrow transplantation in 107 patients with severe aplastic anemia using cyclophosphamide and thoraco-abdominal irradiation for conditioning: long-term follow-up

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