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Outcome of Allogeneic Hematopoietic Cell Transplantation from HLA-Identical Siblings for Severe Aplastic Anemia in Patients Over 40 Years of Age

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Patients with severe aplastic anemia (SAA) over 40 years of age are often not offered treatment with hematopoietic cell transplantation (HCT) because of concerns about treatment-related morbidity or mortality. To evaluate this risk, we analyzed outcomes after allogeneic HCT from HLA-identical sibling donors for all older patients with SAA at our center since 1988. The 23 consecutive patients ranged in age from 40 to 68 years. The conditioning regimen was cyclophosphamide (200 mg/kg) and horse antithymocyte globulin. Methotrexate and cyclosporine were given for postgrafting immunosuppression. The cumulative incidences of grades II, III, and IV acute graft-versus-host-disease were 30%, 4%, and 0%, respectively; that for chronic GVHD was 26%. With a median follow-up of 9.1 years, overall survival was 65%. Documented infections within 1 month before HCT were significantly associated with risk of early treatment-related mortality ($P < .001$). The median time to discontinuation of posttransplant immunosuppression was 6.2 (range: 5.9-92.0) months. Three patients developed superficial basal cell carcinoma between 5.5 and 15 years after HCT. Our data favor a practice of extending HLA-identical sibling HCT for treatment of SAA in patients older than 40 years of age who are without significant medical comorbidities.

Biol Blood Marrow Transplant 16: 1411-1418 (2010) © 2010 American Society for Blood and Marrow Transplantation

KEY WORDS: Aplastic anemia, Older age, Human leukocyte antigen-matched bone marrow transplantation, Cyclophosphamide/antithymocyte globulin conditioning

INTRODUCTION

Severe aplastic anemia (SAA) is a potentially fatal disease characterized by acquired pancytopenia and the absence of hematopoietic elements in the marrow without excess blasts [1]. Hematopoietic cell transplantation (HCT) from a human leukocyte antigen (HLA)-matched donor offers definitive curative treatment [2,3]. For younger SAA patients, long-term survival of approximately 90% has been observed after bone marrow (BM) transplantation from HLA-identical siblings after conditioning with cyclophosphamide and antithymocyte globulin (Cy + ATG) followed by postgrafting immunosuppression with methotrexate and cyclosporine (MTX + CsA) [2,4-6]. Immune suppressive therapy (IST) with ATG and CsA has been used successfully for SAA patients who were not considered candidates for HCT or lack a suitable donor [7-12].

Many transplant center guidelines restrict allogeneic HCT to SAA patients younger than 40 years of age because of concern about the increased risk of treatment-related morbidity and mortality in older patients [13-15]. In a previous study from our Center, long-term survival after HLA-matched related HCT for SAA patients older than 40 years of age was 36% and not significantly different from that of

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Financial disclosure: See Acknowledgments on page 1417.

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Received March 5, 2010; accepted April 3, 2010

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1083-8791/$36.00
doi:10.1016/j.bbmt.2010.04.005

patients treated with IST alone [16]. An analysis by the European Bone Marrow Transplantation (EBMT) SAA working party showed that IST was superior to HCT for SAA patients older than 40 years of age [17]. More recently, an EBMT analysis showed that with Cy 200 mg/kg with or without ATG conditioning, overall survival (OS) at 4 years after HCT was 60% for SAA patients older than 30 years of age; for a smaller cohort of patients receiving a fludarabine (Flu)-based regimen, OS at 4 years was 77% [18]. These results suggest that the outcome of HCT for treatment of SAA in older patients has improved in recent years. Despite the improved survival, concern persists that the conventional Cy 200 mg/kg conditioning regimen may cause excessive toxicity in older SAA patients. Most patients included in the historical studies did not receive what is currently considered the optimal SAA transplant regimen, which consists of Cy + ATG conditioning and MTX + CsA post-grafting immunosuppression.

In the current study we report outcomes for all consecutive patients with SAA above the age of 40 year who had HCT from HLA-identical siblings at a single transplant center from 1988 to 2008. We also identify new pretransplant risk factors that may help to select more suitable HCT candidates among older patients with SAA.

PATIENTS AND METHODS

We retrospectively reviewed the clinical records of all 23 consecutive SAA patients who were older than 40 years and had HCT from HLA-identical siblings at the Fred Hutchinson Cancer Research Center (FHCRC) between July 1, 1988, and October 1, 2008. The protocol was approved by the FHCRC institutional review board (IRB). All patients signed consent forms approved by the IRB. Results are reported as of October 1, 2009.

Patient characteristics are listed in Table 1. The median age of patients was 49 years (range: 40-68 years). The ethnic and racial representation was 5 Hispanic/Latino, 2 Asian, 15 White, and 1 multiple race. The pretransplant performance status was not formally documented. Four patients had infections because of invasive fungal, Gram-negative bacterial, or viral pathogens within 1 month before the start of conditioning, requiring hospitalization for treatment (Table 2). The conditioning regimen consisted of intravenous (i.v.) Cy, 50 mg/kg/day given for 4 consecutive days, and i.v. horse ATG (ATGAM), 30 mg/kg/day given 12 hours after each of the first 3 Cy doses (based on adjusted ideal body weight). Two patients did not receive ATG, 1 because of a positive skin test, indicating severe allergy, and 1 because of drug unavailability. BM was infused 36 hours after the last dose of Cy. Two patients received granulocyte-colony stimulating factor (G-CSF) mobilized donor peripheral blood stem cells (PBSCs). For patient number (PN) 15, PBSCs were used to facilitate more rapid neutrophil recovery after transplant, because the patient had progressive pulmonary aspergillosis before the transplant. PN 17 received Cy + ATG and 2 Gray total body irradiation (TBI) conditioning and PBSCs because he had late graft rejection after the first Cy + ATG HCT given 9 months earlier, at the age of 39 years, from the same donor. Standard MTX/CsA was given as previously reported [2,16]. If there was no evidence of graft-versus-host disease (GVHD), CsA prophylaxis was discontinued at 6 months after HCT. All patients received post-HCT prophylaxis for bacterial, fungal, and viral organisms and were monitored as per current FHCRC standard practice.

Table 1. Pretransplant Characteristics of Patients in the Study

Patients, n	23
Age, in years, median (range)	49 (40-68)
Sex (F/M), n	11/12
Donor-recipient sex mismatched, n (%)	11 (48%)
Major ABO mismatched, n (%)	4 (17%)
ANC on admission, median cells/μL (range)	160 (0-1900)
Platelet count on admission, median × 10^9/L (range)	7.5 (1-49)
Hematocrit on admission, median % (range)	22 (16-30)
Patients with very severe aplastic anemia (ANC <200 cells/μL), n (%)	15 (65%)
Transfused patients prior to HCT	
Red blood cell, n (%)	23 (100%)
Platelet, n (%)	23 (100%)
History of pre-HCT immune suppressive treatment, n (%)	11 (48%)
Median duration of aplastic anemia prior to HCT, months (range)	3 (1-46)
CMV serostatus of patients	
Recipient +/donor +, n (%)	13 (57%)
Recipient +/donor –, n (%)	7 (30%)
Recipient –/donor +, n (%)	2 (9%)
Recipient –/donor –, n (%)	1 (4%)

n indicates number of patients; F, female; M, male; ABO, major blood group antigen type; ANC, absolute neutrophil count; HCT, hematopoietic cell transplantation; CMV, cytomegalovirus.

Donor chimerism was evaluated in either peripheral blood T cells and granulocytes, or in nucleated BM cells on approximately day 80 and/or 1 year after HCT using polymerase chain reaction-based amplification of variable-number tandem repeat or short-tandem repeat sequences unique to donors and recipients, or by fluorescein in situ hybridization for X and Y chromosomes in cases when patients and donors were sex mismatched. Mixed chimerism was defined as >5% and <95% donor chimerism in at least 1 cell lineage.

Median, range, and proportions were used to summarize descriptive data, as appropriate. OS was estimated using the Kaplan-Meier method with time to death as the primary outcome and censoring at last follow-up. Probabilities of acute and chronic GVHD (aGVHD, cGVHD), time to discontinuation of immunosuppression, and rejection were calculated using cumulative incidence estimates. Time to the event of interest was the primary outcome. Death was treated

Table 2. Relevant Clinical Data for SAA Patients after HCT

Patient Number (PN)	Age (Years)	Infused Cell Dose TNC $\times 10^8$/kg	aGVHD Day Onset/Grade	cGVHD (Day of Onset)	Duration of IST Post- HCT (Months)	Fluid Overload (Day of Onset)	Pneumonia (Day of Onset)	Bacteremia (Day of Event)	% Donor Chimerism (day +80)	Outcome (Day/Year of Last Follow-up after HCT)
1	46	3.11	+16 / III	+107	until death	—	+152	+94	99 BM	Death (d+179)
2	46	3.56	NE	NE	until death	+1	—	—	NE	Death (d+3)
3	42	2.18	—	—	6	—	—	—	NA	CR (19.2 yr)
4	42	1.67	—	—	6	−3	+220	—	98 BM	CR (16.2 yr)
5	40	4.36	NE	NE	until death	−3	—	−7	NE	Death (d+6)
6*	51	1.31*	+80* / II	—	9.3	—	—	—	100 BM*	CR (16.2 yr)
7	48	1.92	—	—	6	—	—	—	75 BM	CR (15.1 yr)
8	41	2.18	—	—	6.5	—	—	—	90 BM (1 yr)	CR (15.0 yr)
9	59	1.96	+25 / II	NE	until death	—	+76	+45, +65	NA	Death (d+83)
10	49	2.71	+43 / II	+119	until death	—	—	+147	NA	Death (d+223)
11	55	1.85	+19 / II	—	7.5	—	—	—	100 BM	CR (14.1 yr)
12	45	2.36	+69 / II	—	6	—	+256	—	100 BM	CR (8.1 yr)
13	56	4.84	—	+85	92	—	—	—	99.5 BM	CR (12.9 yr)
14	59	2.30	+24 / II	+92	23	—	—	+32, +210	100 BM	CR (9.1 yr)
15†	53	8.74†	NE	NE	until death	−1	−12	—	NE	Death (d+2)
16	42	4.60	—	—	6	—	—	—	59.5 CD3	CR (7.0 yr)
17†	40	22.34†	—	+162	80	0	—	—	100 BM (1 yr)	CR (6.7 yr)
18	52	1.68	—	—	6	—	—	−12	95-99 CD3	CR (7.2 yr)
19	63	2.37	+41 / II	+258	64	—	—	+51, +67	85 CD3	CR (6.3 yr)
20	57	2.45	—	—	6	—	+10	—	67 CD3	CR (5.0 yr)
21	49	2.25	NE	NE	until death	—	−22, +14	−17, −4	NE	Death (d+20)
22	68	2.05	NE	NE	until death	0	−5	+8	NE	Death (d+11)
23	50	1.85	+24 / II	—	7	—	—	—	18 CD3	CR (0.9 yr)

1 yr indicates donor chimerism at 1 year after HCT (was not assessed at day 80); aGVHD/cGVHD, acute/chronic graft-versus-host disease; BM, bone marrow chimerism; CD3, T cell chimerism (sorted from peripheral blood) and was performed only for PN 16, 18, 19, 20, and 23; CR, complete remission (and alive); HCT, hematopoietic cell transplantation; IST, immunosuppressive therapy for treatment of GVHD; NA, not assessed; NE, not evaluable; SAA, severe aplastic anemia; TNC, total nucleated cells.

Day 0 is the day of the HCT infusion.

Donor chimerism was assessed by X or Y chromosome FISH probes or donor specific short-tandem repeat sequence PCR analysis. Donor CD33 chimerism at day 80 was 100% for PN 18, 19, 20, and 23 and was 98% for PN 16.

*PN 6 had graft rejection on day +28 after first HCT (this was considered the primary outcome after HCT); a third HCT was successfully performed from the same donor 75 days after first HCT. PN 6 developed aGVHD on day +80 after third HCT and had complete donor chimerism.

†PN15 and PN17 received G-CSF-mobilized peripheral blood stem cells.

as a competing risk event for all outcomes; in addition, graft rejection was a competing risk for GVHD and discontinuation of immunosuppression [19]. Associations of pretransplant risk groups with survival were evaluated using log-rank tests. All reported *P*-values are 2-sided, with a significance level of .05.

RESULTS

Engraftment

Outcomes after HCT are summarized in Table 2, which lists patients by PN in chronological order. The overall rate of graft rejection was 4.3%. Seventeen of 18 patients who survived beyond 3 weeks after HCT showed sustained engraftment, documented by increasing blood cell counts and BM cellularity, and by assessment of donor chimerism. The absolute neutrophil count surpassed 500/μL at a median of 24 (17-28) days after HCT. One patient (PN 6) had graft rejection by day 28 after HCT after infusion of BM with a total nucleated cell (TNC) cell dose of 1.31×10^8/kg. A second HCT from the same donor with Cy + ATG reconditioning failed. Sustained engraftment was established after a third HCT from the same donor following reconditioning with a CD3-specific monoclonal antibody combined with high-dose methylprednisolone [20].

Donor chimerism was assessed on day 80 after HCT in 13 patients (Table 2). The median donor chimerism was 99.5% (range: 75%-100%) in BM (n = 8) and 100% (range: 98%-100%) in CD33 granulocyte PBSCs (n = 5). The median CD3 T cell donor chimerism was 67% (range: 18-99%, n = 5). Mixed chimerism was seen in 38% of evaluable patients. Donor chimerism increased at 1 year after HCT in all evaluable mixed chimeras.

GVHD

Eight patients (35%) developed grades II (30.4 %) and III (4.3%) aGVHD; the median onset of aGVHD was day 25 (16-69) after the first HCT (Table 2 and Figure 1a). Grade IV GVHD was not observed. aGVHD improved in all patients after treatment with methylprednisolone and continued administration of CsA. In addition, 2 patients received beclomethasone and budesonide for GVHD treatment. The gastrointestinal tract was affected by aGVHD in 7 cases.

The cumulative incidence of cGVHD at 2 years after HCT was 26%, with onset requiring systemic immunosuppressive treatment at a median of 3.7 (2.8–8.5) months after HCT (Table 2 and Figure 1b). The median time to discontinuation of immunosuppressive treatment after HCT was 6.2 (5.9–92.0) months among the patients who were able to discontinue immunosuppression before death or rejection (Figure 1c).

Causes of Death/Fatal Infections

Five patients (22%) died before engraftment between days +2 and +20 after HCT (Table 2). PN 15 died on day +2 because of preexisting disseminated invasive aspergillosis. PN 2 developed progressive pulmonary edema during conditioning coincident with a central venous catheter infection and fever on day −3, and died with cardiac tamponade on day +3. PN 5 developed *Klebsiella* bacteremia on day −7, pulmonary edema during conditioning, followed by multiorgan failure; she died on day +6, and the autopsy showed disseminated candidiasis. PN 22 had recurrence of an incompletely treated Klebsiella pneumonia on day −5, developed septic shock with *Staphylococcus aureus* on day +8, and died on day +11. PN 21 who had common variable immunodeficiency before the diagnosis of SAA, developed progressive invasive pulmonary aspergillosis and cytomegalovirus (CMV) pneumonia 3 weeks before HCT, and died on day +20 with acute respiratory distress syndrome and *Burkholderia cepacia* pneumonia. Two of these 5 patients had received 6 to 9 months of prednisone, CsA, and ATG before HCT.

The 4 patients who had documented infections because of invasive fungal, Gram-negative bacterial or viral pathogens within 1 month before the start of conditioning died within 20 days after HCT. With the exception of PN 18, who developed a coagulase-negative *Staphylococcus epidermidis* central venous catheter infection on day −12, none of the 18 patients surviving beyond day +20 had significant infections during the month before HCT. Thus, documented invasive fungal, bacterial, or viral pneumonia or Gram-negative bacteremia within 1 month before HCT was significantly associated with risk of early traeatment-related mortality, $P < .001$ (log-rank test). In addition, there were 4 patients with documented bacteremia or invasive fungal infection successfully treated more than 1 month before HCT. None of these 4 patients died after HCT. We found no association between duration of AA before HCT and mortality after HCT.

Three patients died on days 83, 179, and 223 after HCT, respectively. PN 9 died from respiratory syncytial virus (RSV) pneumonia and adenovirus nephritis that developed during prednisone treatment for aGVHD. PN 1 died with a mixed *Pseudomonas aeruginosa* and CMV pneumonia during immunosuppressive treatment for cGVHD. PN 10 died with polymicrobial sepsis associated with severe cGVHD.

OS

With a median follow-up of 9.1 years (range for surviving patients: 0.9-19.2 years), OS was 65% at 10 years after HCT (Figure 1d). All 15 surviving patients remain in complete remission of SAA with normal

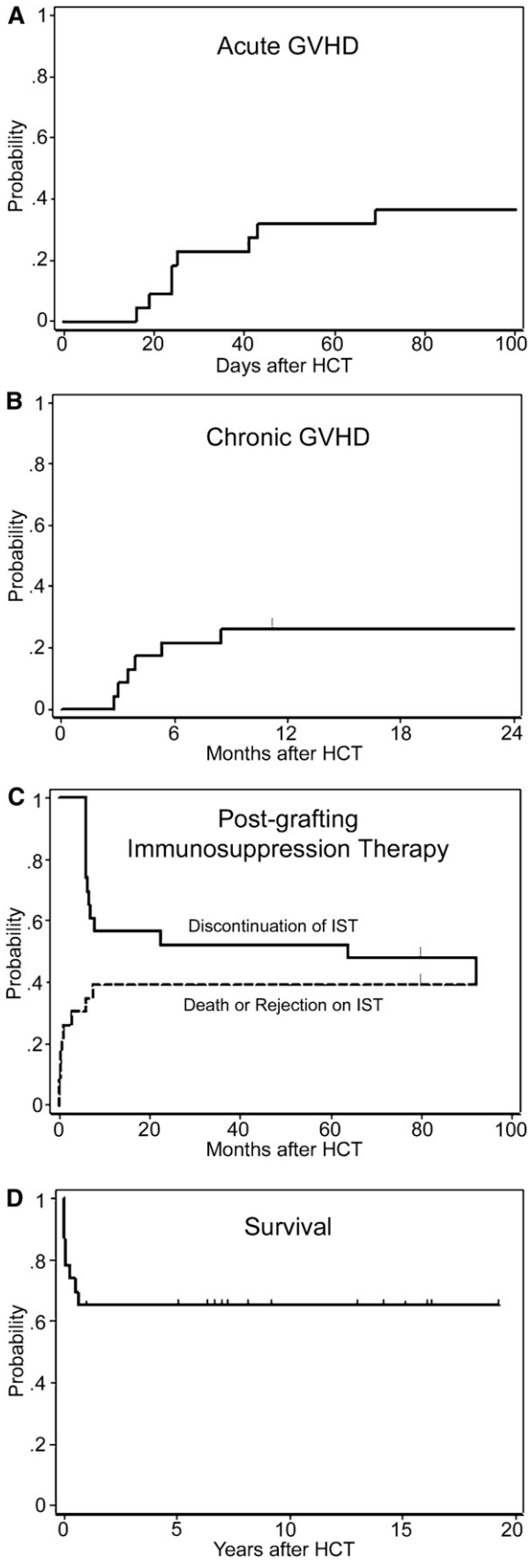

Figure 1. (A) Cumulative incidence and onset of aGVHD (30.4% grade II, 4.3% grade III). (B) Cumulative incidence and onset of cGVHD (26%). (C) Upper curve shows declining proportion of patients continuing postgrafting IST, lower curve shows cumulative incidence of death or rejection while on immunosuppression therapy. Area between curves represents proportion of patients who are alive, rejection free, and on immunosuppression therapy. (D) OS after HLA-identical HCT for SAA for all patients (n = 23) over 40 years of age (65% survival at 10 years). Tick marks indicate time of last follow-up.

blood cell counts, and do not require transfusion or G-CSF support. No deaths have occurred beyond day 223 after HCT. OS was 79% when patients with documented active or progressive fungal, bacterial, or viral pneumonia or Gram-negative bacteremia within 1 month before conditioning for HCT were excluded.

Nonfatal Infections

Seven patients (30%) had significant but nonfatal infections after HCT (Table 2). Invasive aspergillus pneumonia was diagnosed in 1 patient on day +10. Four patients developed bacteremia with Gram-positive cocci on days +32, +45, +67, and +147, respectively; a second episode occurred in 1 patient at 7 months after HCT. Three patients developed bacteremia with Gram-negative rod organisms on days +51, +65, and +94, respectively, concurrent with treatment for gastrointestinal aGVHD. One patient was diagnosed with mycoplasma pneumonia at +220 days after HCT. All of these infections were successfully treated.

CMV Reactivation

Eleven patients (50% of those at risk) had CMV reactivation detected by testing for antigen or DNA, with onset between 24 and 256 (median, 57) days after HCT. Two patients developed CMV pneumonia on days 152 and 256 after HCT, respectively (Table 2). Ten of 11 patients (91%) with CMV reactivation after HCT had complete resolution with antiviral treatment.

Fluid Overload

Six patients (26%) developed fluid overload requiring specific medical treatment either during the conditioning regimen or within the first week after HCT (Table 2). Three deaths early after HCT were associated with concurrent pretransplant infections (PN 5, 15, and 22) as described earlier; clinical or autopsy findings were not consistent with Cy-induced cardiotoxicity. Two patients developed transient diffuse pulmonary edema during ATG and HCT infusion, respectively. For both patients, contemporaneous echocardiograms showed normal left ventricular ejection fraction, and symptoms resolved with treatment. As described before, PN 2 developed pulmonary edema and cardiac tamponade and died on day +3. An

autopsy was not obtained. Cy-induced cardiotoxicity could not be excluded as a possible cause of death.

Delayed Posttransplant Complications

Three patients (PN 11, 14, 13) developed avascular bone necrosis at 2, 3, and 9 years after HCT, respectively. The first patient had received a total of 6 months of prednisone treatment and the next 2 patients received immunosuppressive treatment for 23 and 77 months after HCT, respectively. Three patients (PN 3, 11, and 13) developed superficial basal cell carcinomas with successful resection at 5.5, 8.7, 8.8, and 15 years after HCT, respectively.

DISCUSSION

Allogeneic HCT provides curative therapy for patients with SAA, but many transplant centers restrict its application to patients younger than 40 years of age because of concerns about toxicity of the Cy + ATG conditioning regimen and the risk of GVHD. Our retrospective study clearly indicates that HCT from HLA-matched sibling donors can be successfully extended to SAA patients older than 40 years (the median age of patients in this study was 49 years). However, systemic infections at the time of HCT identify poor-risk candidates with a very high risk of early mortality.

The observed long-term OS of 65% at 10 years compares very favorably with recently published results for SAA patients treated with IST, where long-term survival in patients above 50 years of age was less than 40% [9]. The current results are particularly significant because preceding IST had been unsuccessful in 48% of our patients.

A survival benefit in favor of HCT over IST was consistently observed in historical studies of SAA patients, but this benefit was limited to patients younger than age 40 years. Older SAA patients had equivalent or worse OS after HCT compared to IST [16,17]. These observations were likely influenced by the fact that many patients did not receive what is now considered the optimal transplant conditioning with Cy + ATG and postgrafting GVHD prophylaxis with MTX + CsA.

A recent multicenter EBMT study of HCT with HLA-identical sibling donors showed an OS rate of 71% with a median follow-up of 4 years after a Flu-based conditioning regimen in 21 SAA patients >40 years of age [18]. OS was 60% at 5 years among 239 SAA patients >30 years of age who receiving the standard conditioning regimen of Cy 200 mg/kg with or without ATG. Despite the improved survival outcome compared to historical studies, concern persisted that the conventional Cy 200 mg/kg-based conditioning regimen may cause excessive toxicity in older SAA patients. It is unclear, however, whether the Flu-based regimen is superior to Cy + ATG, because patients in the EBMT study were not randomized between the treatment arms. In addition, most patients did not receive ATG with Cy, 34% received mobilized blood cell grafts, and the postgrafting immunosuppressive regimen was not uniform.

In our current study, the incidence of graft rejection was low. One patient had primary graft rejection but had sustained engraftment after a third HCT, and 1 patient had secondary graft rejection at age 39 years and had successful second HCT at age 40 years (for which data are shown). Graft rejection was previously associated with transfusions given before HCT. Although all patients had received multiple transfusions before HCT, nearly all of these blood products had been depleted of leukocytes or irradiated [21,22]. The median time from diagnosis to HCT was 3 months, which may have also contributed to the observed low incidence of graft rejection.

Mixed hematopoietic chimerism at 80 days after HCT was not associated with graft rejection. Thirty-eight percent of evaluable patients had mixed chimerism at day 80, and all mixed chimeras had a subsequent increase in donor chimerism. There may have been additional patients with mixed T cell chimerism among those with >95% donor BM chimerism, but prior to March 2001 PB T cell chimerism was not routinely assessed.

The observed cumulative incidences of aGVHD and cGVHD in our study (35% and 26%, respectively) were comparable to that reported previously in studies with younger patients with SAA [6]. Furthermore, the incidence of grade III GVHD was only 4.3% and grade IV GVHD was not observed. It is very likely that the use of ATG during conditioning prevented both graft rejection and GVHD. In most cases, GVHD responded promptly to a short course of prednisone therapy.

The most striking finding in this study was that an active infection within 1 month before HCT, whether because of a Gram-negative bacterial, a fungal, or a viral pathogen, was associated with early death after HCT. The OS at 10 years for patients without documented pneumonia or Gram-negative bacteremia during the month before HCT was 79%. Patients with a history of infection successfully treated more than 1 month before HCT were not at an increased risk for early mortality after HCT. These findings suggest that to reduce the risk of early death after HCT, SAA patients should either proceed to HCT before onset of pneumonia or Gram-negative bacteremia, or delay HCT until infection has completely resolved. On the other hand, the primary reason for proceeding to HCT with an active infection was the concern that infection would be fatal unless hematopoietic function could be restored. Our results support the findings of retrospective studies, which showed that patients with invasive fungal infections or RSV infections

within the month before HCT have an increased risk of mortality [23]. In addition, severe neutropenia prior to HCT in patients with myelodysplastic syndrome was associated with increased infectious mortality after HCT [24].

We observed that 26% of patients developed pulmonary edema or fluid overload between days −3 to +1. Detailed review suggested that this complication was exacerbated primarily by concurrent systemic infection and was not directly because of Cy-induced cardiomyopathy. The development of systemic infection immediately before HCT was associated with a high risk of fatal cardiopulmonary toxicity during or after conditioning. Careful cardiac screening before transplantation and frequent monitoring of fluid status during and after administration of Cy + ATG might help to minimize the risk of toxicity, especially in older patients.

The incidence of second malignancies (3 cases of basal cell carcinoma) in our older cohort was not significantly higher than that observed in younger patients [4]. Although basal cell carcinoma is not a life-threatening complication, the data reinforce the need to monitor patients for second malignancies after HCT. Our results provide further support for HCT as treatment for SAA, because nontransplant IST is associated with a higher risk of developing fatal subsequent malignancies, particularly in older patients [9]. The 3 cases of avascular hip necrosis emphasize the need to improve the prevention and treatment of GVHD and to reduce the duration of steroid-based treatment.

In conclusion, although this was a retrospective study that included several patients that were transplanted more than 15 years ago, our results suggest that older patients with AA who have an HLA-matched sibling and who are without significant medical comorbidities should proceed to BM transplantation promptly for definitive curative therapy. Patients who have compromised clinical conditions such as cardiomyopathy or systemic infection during the month before HCT are not suitable candidates for the use of Cy + ATG as a conditioning regimen. A history of active or very recent infections adversely affects HCT outcome and should be taken into account when making treatment decisions for older patients with SAA.

ACKNOWLEDGMENTS

Financial disclosure: This work was supported by grants HL36444, CA15704, and AI067770 National Institutes of Health, Bethesda, MD, USA. D.S. is a fellowship recipient "Assegno di Ricerca," funded by Regione Piemonte, Italy. The authors thank the patients and their families, the transplant and long-term follow-up teams, the referring physicians, and the database managers, particularly Gary Schoch, for their invaluable help with the study, and Bonnie Larson, Helen Crawford, and Sue Carbonneau for help with manuscript preparation.

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