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Hematopoietic stem cell transplantation for high-risk adult patients with severe aplastic anemia; reduction of graft failure by enhancing stem cell dose

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Background and Objectives. The main causes of failure after allogeneic hematopoietic stem cell transplantation (HSCT) in patients with severe aplastic anemia (SAA) are graft-versus-host disease (GVHD), infection and graft failure, often exacerbated by large numbers of transfusions and prolonged disease duration before transplant. This study retrospectively analyzes the outcome and factors related to survival or graft failure in high-risk patients with SAA receiving HSCT in our institution.

Design and Methods. Between January 1995 and December 1999, 40 consecutive adult patients who were multi-transfused (more than 40 units of red blood cells ± platelets) and/or had a 3 years or longer period prior to transplant were enrolled. Their median age was 27.5 years (range, 16 to 43) and 21 (52.5%) were women. All donors were human leukocyte antigen (HLA)-matched siblings. Before transplant, 29 patients (72.5%) received a course of antithymocyte globulin (ATG) and cyclosporin A (CsA). The median interval from diagnosis to transplant was 59 months (range, 2 to 216). The median number of transfusions was 115 units (range, 10 to 480). All patients received a conditioning regimen of cyclophosphamide, ATG, and procarbazine. Our patients received either bone marrow (BM) alone (n=20) or BM+peripheral blood stem cells (PBSC) (n=20) as a stem cell source. T-cells of PBSC were depleted using the CD34 enrichment method. GVHD prophylaxis consisted of CsA and short-term methotrexate.

Results. In the BM+PBSC group, neutrophil recovery to 0.5×10^9/L and platelet recovery to 20×10^9/L were achieved more rapidly than in the BM group (p=0.005 and 0.039, respectively). The incidences of graft failure, grade II to IV acute GVHD, and chronic GVHD were 22.5%, 12.8% and 23.1%, respectively. Graft failure occurred in 2 of 20 patients (10%) receiving BM+PBSC and in 7 of 20 (35%) receiving BM alone (p=0.069). Seven of 9 patients who had graft failure received a booster treatment and recovered normal marrow function. GVHD incidence was comparable between the BM+PBSC and BM groups. Six patients (15%) died from graft failure (n=2), interstitial pneumonia (n=2), cyclophosphamide-induced heart failure (n=1), and chronic GVHD followed by pneumonia (n=1). The Kaplan-Meier estimate of survival was 83.7% with a median follow-up duration of 40.5 months (range 8-67). In multivariate analysis only chronic GVHD adversely influenced survival (p=0.042).

Interpretation and Conclusions. These results suggest that HSCT is an effective treatment for multi-transfused SAA patients with prolonged disease duration. It is highly possible that the infusion of a large number of stem cells leads to a reduction of graft failure and a faster speed of engraftment. Booster treatment is successful in achieving engraftment in patients with graft failure.

Key words: severe aplastic anemia, hematopoietic stem cell transplantation, graft failure, multi-transfusion, long disease duration

Results of hematopoietic stem cell transplantation (HSCT) for patients with acquired severe aplastic anemia (SAA) have improved considerably over the last decades.[1] However, a large retrospective analysis suggests that it is the incidences of graft-versus-host disease (GVHD) and interstitial pneumonia that have decreased with little change in graft failure.[2] The *Registry of the European Blood and Marrow Transplantation* (EBMT) shows that for patients receiving human leukocyte antigen (HLA)-identical sibling grafts, there has been a modest reduction of graft failure over time: 18% before 1980, 12% from 1980 to 1990, and 11% from 1991 to 1998 (p=0.12).[3] Older patients are at higher risk of graft failure, for reasons not completely understood. Other pretransplant factors associated with graft failure are exposure to large numbers of transfusions and long duration of disease, which are obviously correlated.[4] Exposure to multiple transfusions sensitizes the recipient to histocompatible antigens.[5,6]

Over several decades, various transplant strategies have been introduced to decrease graft failure, with mixed success.[2,7,8] In our high-risk patients, an intensified pretransplant conditioning with or without increas-

Table 1. Patient- and disease-related variables of the high-risk patients receiving HLA-identical sibling transplantation for severe aplastic anemia between 1995 and 1999.

Patient no.	*Stem cell source*	*Age yrs.*	*Sex (R/D)**	*Blood type*	*Donor age yrs.*	*Interval mos.*	*Transfusions no.*	*Prior IST°*	*Refractory to platelets*	*Others*
365	BM+PBSC	31	F/F	B/O	40	120	200	+	+	
389	BM+PBSC	33	M/M	B/B	18	36	150	+	+	
397	BM+PBSC	34	F/M	B/A	40	108	250	+	+	
409	BM+PBSC	34	M/M	A/A	38	72	480	+	+	organ dysfunction
426	BM+PBSC	20	F/M	O/O	24	120	100	+	-	familial transfusion
427	BM+PBSC	32	F/F	A/AB	41	96	100	+	-	
433	BM+PBSC	24	M/M	B/B	26	4	10	+	-	familial transfusion
435	BM+PBSC	29	M/F	A/B	37	84	100	+	-	
458	BM+PBSC	23	M/M	A/A	32	11	50	+	-	multiple folliculitis
478	BM+PBSC	28	M/F	O/O	40	9	120	-	-	
479	BM+PBSC	24	F/F	O/O	29	22	50	+	-	
516	BM+PBSC	37	M/M	A/A	39	11	50	+	-	
529	BM+PBSC	23	M/F	O/A	25	84	250	-	+	organ dysfunction
540	BM+PBSC	31	F/M	A/B	24	132	200	+	+	organ dysfunction
638	BM+PBSC	27	F/M	O/O	32	72	300	-	+	organ dysfunction
787	BM+PBSC	24	M/M	B/A	22	96	450	-	+	organ dysfunction
814	BM+PBSC	43	F/M	A/O	46	216	230	+	+	
837	BM+PBSC	43	F/F	B/B	44	36	110	-	-	
842	BM+PBSC	24	F/M	A/O	21	90	300	+	+	organ dysfunction
810	BM+PBSC	27	M/M	O/O	43	2	130	-	-	pneumonia, VSAA[#]
197	BM	23	F/F	O/O	25	108	150	-	-	
208	BM	35	F/M	A/A	48	15	100	+	-	
212	BM	25	M/M	A/A	27	180	100	-	+	infection
217	BM	21	F/F	B/A	14	36	60	+	-	
239	BM	18	M/M	O/B	17	5	50	-	-	familial transfusion
241	BM	16	M/F	B/B	14	48	150	-	+	
245	BM	33	M/M	B/B	28	44	150	+	+	
260	BM	26	F/F	A/A	25	21	200	+	+	
265	BM	29	F/M	B/B	23	68	210	+	+	
287	BM	33	M/F	B/B	40	72	90	+	-	
293	BM	22	M/F	O/O	21	36	80	+	-	
328	BM	20	F/F	B/B	38	132	40	+	-	familial transfusion
341	BM	23	M/F	B/O	27	12	250	+	+	VSAA[#]
360	BM	29	F/M	AB/A	34	216	200	+	+	organ dysfunction
377	BM	28	F/M	A/AB	27	74	300	+	+	
382	BM	25	M/F	A/A	30	60	60	-	-	
434	BM	29	M/M	O/O	27	48	65	+	+	
624	BM	16	F/M	A/A	18	23	80	+	+	
688	BM	41	F/M	A/B	29	58	40	+	-	
812	BM	29	F/M	A/B	26	53	100	+	-	
Median		27.5			27.5	59	115			

**Recipient/donor; °Immunosuppressive therapy consisted of antilymphocyte globulin and cyclosporin A; [#]very severe aplastic anemia, defined by an absolute neutrophil count < 0.2×10^9/L; mos. = months, yrs. = years.*

ing donor cells in the infusion was used in an attempt to reduce graft failure. The aim of the present study was to analyze the outcome and factors related to graft failure and survival in patients with long disease duration and/or multiple transfusions receiving a transplant from an HLA-identical sibling.

Design and Methods

Patient selection

Between January 1995 and December 1999, 40 consecutive adult patients who were multi-transfused (more than 40 units of red blood cells ± platelets) and/or had a 3 years or longer period prior to transplantation were transplanted from an HLA-identical sibling in our institution. SAA was defined according to the criteria of the *International Aplastic Anemia Study Group*.[9] The characteristics of the patients, disease and previous treatments are shown in Table 1. The patients' median age was 27.5 years (range, 16 to 43); 21 (52.5%) were women and 19 were men. In all cases the etiology of the aplasia was unknown. The median interval between diagnosis of SAA and transplantation was 59 months (range, 2 to 216). The median number of transfusions was 115 units (range, 10 to 480). When exact data were not available, the numbers of transfusions were reported to make a roughly minimum estimate. Four patients (10%) received transfusions from family members without leukodepletion or irradiation. Before transplantation, twenty-nine patients (72.5%) received one or two courses of antithymocyte globulin (ATG) and cyclosporin A (CsA). Twenty patients (50%) were refractory to random donor platelets. Our patients received filtered blood components approximately after 1989. In 1990 we began to take two steps including both leukodepletion and irradiation. Sixteen patients (40%) were ABO mismatched with their donor.

The combination of donor/patient sex was female/male in 8 patients (20%). Three patients showed clinical signs of infection in the week before transplantation. Seven patients (17.5%) had organ dysfunctions related to heavy transfusions.

Transplant characteristics

Conditioning regimen and GVHD prophylaxis

The preparative regimen used in the 40 patients consisted of cyclophosphamide (CY) 50 mg/kg/day once daily i.v. for 4 days (total dose 200mg/kg), ATG (1.25 mg/kg/day once daily i.v. for 3 days, rabbit type, Pasteur Merieux, France), and procarbazine (6.25 mg/kg/day p.o. in divided doses daily for 6 days).[10] The primary prophylactic regimen for GVHD was a combination of CsA and methotrexate (MTX). CsA was given as a continuous i.v. dose of 3 mg/kg/day until 21 days post-BMT and then p.o. at 6 mg/kg/day, while MTX was given intravenously at a dose of 10 mg/m^2 on days 1, 3, 6, and 11. The severity of acute and chronic GVHD was assessed according to previously described criteria.[11,12]

Stem cell sources

Twenty patients received unmanipulated bone marrow (BM) alone. Donor BM cells were obtained under general anesthesia by multiple aspirations from both iliac crests and infused fresh without manipulation. In the other 20 patients, unmanipulated BM cells were infused following CD34$^+$ enriched peripheral blood stem cell (PBSC) transfusion. G-CSF (10 μg/kg/day) was administered to donors by subcutaneous injection and continued for 5 days. Two leukaphereses were performed on days 5 and 6 of G-CSF administration. The PBSC preparations were depleted of T-lymphocytes using an immuno-adsorption biotin-avidin column (Ceprate SC, Cell pro, Bothell, WA, USA)[13] in 15 patients. After January 1999, magnetic cell sorting was performed to purify PBSC with adequate recovery of CD34$^+$ cells in the remaining 5 patients (CliniMACS system, Miltenyi Biotec, Bergisch Gladbach, Germany).[14] BM harvesting was performed 48 hours after the last leukapheresis at a volume of 15mL per kg of recipient body weight.

Supportive care

All patients were treated in rooms with laminar airflow isolation and received oral non-absorbable antibiotics and low microbial diet for gut decontamination. Trimethoprim-sulfamethoxazole was administered for *Pneumocystis carinii* prophylaxis. Patients with an absolute neutrophil count (ANC) of less than 0.5×10^9/L and body temperatures exceeding 38.3°C were empirically treated with broad-spectrum antibiotics. Subcutaneous G-CSF (5 μg/kg/day) was started on the 7th day post-PBSC infusion and continued until the ANC reached 1.0×10^9/L on 3 consecutive days.

Engraftment

The time needed for hematopoietic recovery was measured at an ANC $\geq0.5\times10^9$/L and a platelet count $\geq20\times10^9$/L without platelet transfusion. Engraftment was also assessed by routine marrow aspirates. Graft failure was defined as either primary graft failure, ie, absence of hematologic recovery in patients surviving ≥ 21 days after transplantation; or transient engraftment, ie, complete or partial recovery of hematopoiesis of donor origin followed by recurrent pancytopenia with a markedly hypocellular bone marrow in the absence of moderate to severe acute GVHD.[6,15]

Statistical analysis

Results of the study were analyzed as of 31 August 2000. Actuarial probabilities of survival and graft failure were calculated using the Kaplan-Meier product limit method. Patients surviving ≥ 30 days and ≥ 100 days, with engraftment, were considered at risk of acute and chronic GVHD, respectively. Engraftment kinetics of neutrophils and platelets after HSCT were compared using the log-rank test. Univariate analysis was used to test associations between patient-, disease- and treatment-related variables and the probability of GVHD, graft failure and survival. Factors associated with survival and graft failure in these univariate analyses with a p value < 0.2 were entered into a multivariate Cox proportional hazards regression model using a forward stepwise approach. A p value <0.05 after multivariate analyses was considered to be statistically significant. Statistical analyses were performed using SPSS for Windows (Release 9.9.0).

Results

Patients' characteristics and cell counts

The risk factors with regards to engraftment as previously mentioned were not significantly different between the BM and BM+PBSC groups (Table 2). The donors in the BM+PBSC group were older than those in the BM group (p =0.03).

In the BM+PBSC group, the final inoculum contained a median of 4.33 (1.84-6.47)$\times10^8$/kg mononuclear cells, 15.37 (5.73-24.1)$\times10^6$/kg CD34$^+$ cells, and 86.22 (8.96-331.2)$\times10^6$/kg CD3$^+$ cells, while in the patients receiving BM alone, the numbers of mononuclear cells, CD34$^+$ cells, and CD3$^+$ cells were 1.23 (0.88-2.06)$\times10^8$/kg (p <0.001), 2.86 (1.8-4.9)$\times10^6$/kg (p =0.001), and 63.67 (57.0-70.0)$\times10^6$/kg (p =0.801), respectively (Table 3).

Donors tolerated G-CSF treatment well with only low-grade fever, bone pain, and headache, which ceased 2-3 days after marrow harvesting. The bone marrow harvesting procedure was rapid. In most cases it was possible for two operators to collect 1 L of marrow during a 30-min period. Donors reported no untoward side effects after the leukaphereses and marrow harvesting. No donor received platelet transfusions.

Engraftment and graft failure

The median time to recovery of granulocytes to $\geq0.5\times10^9$/L was 13 days (range, 10-49). The median day to achieve a platelet count more than 20×10^9/L without transfusions was 23 (range, 4-125). These times dif-

Table 2. Comparison of patient characteristics according to the type of infused stem cells.

Characteristics	*Total patients (n=40)*		
	BM (n=20)	*BM+PBSC (n=20)*	*p value*
Median patient age (range), years	25.5 (16-41)	28.5 (20-43)	ns
Median donor age (range), years	27 (14-48)	34.5 (18-46)	0.03
Sex of recipient (male/female)	9/11	10/10	ns
Sex of donor/patient, n (%)			
Female/male	5 (25)	3 (15)	ns
Other pairs	15 (75)	17 (85)	ns
ABO type of patient/donor, n (%)			
Match	13 (65)	11 (55)	ns
Major mismatch	5 (25)	6 (30)	ns
Minor mismatch	2 (10)	3 (15)	ns
Preceding transfusions (RBC+platelets)			
Median (range), units	100 (40-300)	140 (10-480)	ns
Median disease duration (range), months	50.5 (5-216)	78 (2-216)	ns
Previous ATG therapy, n (%)	15 (75)	14 (70)	ns
Transfusion from family members prior to transplant, n (%)	2 (10	2 (10)	ns
Refractory to transfusion with platelets from randomly selected donors, n (%)	10 (50)	10 (50)	ns
VSAA, n (%)	1 (5)	1 (5)	ns

Abbreviations; n=number, ns=not significant, VSAA=very severe aplastic anemia.

Table 3. Infused stem cell doses.

	BM alone (n=20)	*BM+PBSC (n=20)*	*p value by T-test*
Mononuclear cells ($\times 10^8$/kg)			
Mean ± SD	1.23 ± 0.27	4.33 ± 1.53	< 0.001
$CD34^+$ cells ($\times 10^6$/kg)			
Mean ± SD	2.86 ± 1.76	15.37 ± 7.02	0.001
$CD3^+$ cells ($\times 10^6$/kg)			
Mean ± SD	63.67 ± 6.51	86.22 ± 78.97	0.801

Table 4. Outcome after booster treatment in patients with graft failure.

Patient	*Conditioning prior to boost*	*Boost cell source*	*After booster treatment*			*Outcome*
			Prophy-laxis	*aGVHD ≥ grade II*	*cGVHD*	
217	TNI+S	PBSC	C	–	+	Alive
239	TNI+S	BM	C	+	–	Alive
241	TNI+S	PBSC	C	+	–	Alive
245	TNI+ATG	BM	C	–	–	Alive
360	ATG	PBSC	C	–	+	Alive
427	No	PBSC	C	–	–	Alive
529	TNI+ATG	PBSC	C	–	–	Alive

Abbreviations; aGVHD=acute graft–versus–host disease; cGVHD = chronic GVHD; TNI = total nodal irradiation; ATG = antithymocyte globulin; BM = bone marrow; PBSC = peripheral blood stem cells; S = steroid; C = cyclosporin A.

fered according to the type of stem cell source; in the BM+PBSC group, neutrophil recovery to 0.5×10^9/L and platelet recovery to 20×10^9/L were achieved more rapidly than in the BM group (Figure 1, p=0.005 and 0.039, respectively). In patients who received BM+PBSC, the median time to granulocyte recovery more than 0.5×10^9/L was 12 versus 17 days in those transplanted with BM alone. The patients receiving BM+PBSC reached a platelet count of 20×10^9/L a median of 17 days after transplantation, whereas the patients receiving BM alone took 25 days.

Graft failure occurred in nine of the 40 (22.5%) patients. One patient had primary graft failure and eight had transient engraftment with loss of the graft between 5 and 24 weeks (median, 8) post-HSCT. The type of infused stem cells had a major impact on the probability of graft failure. Two of the 20 (10%) patients transplanted with BM+PBSC rejected their graft; in contrast, 7 of the 20 patients (35%) receiving BM alone had graft rejection. The difference is strongly suggestive (Figure 2, p=0.069). Seven of 9 patients with graft failure received a booster treatment and are alive with a normal graft function; G-CSF mobilized PBSC was used in 5 patients and pre-boost immunosuppression such as ATG or total nodal irradiation in 6 (Table 4). Two patients who did not receive the booster treatment after graft failure died, one of infection and one of hemorrhage.

Graft-vs-host disease

Eight of the 39 (20.5%) patients at risk developed acute GVHD including 5 with grade II to IV disease. The actuarial probability of ≥ grade II GVHD at four months was 13%. In univariate analysis the only factor associated with a marginal risk of acute GVHD was the donor age (p=0.058). Nine of the evaluable 39 patients (23.1%) developed chronic GVHD with 2 having extensive dis-

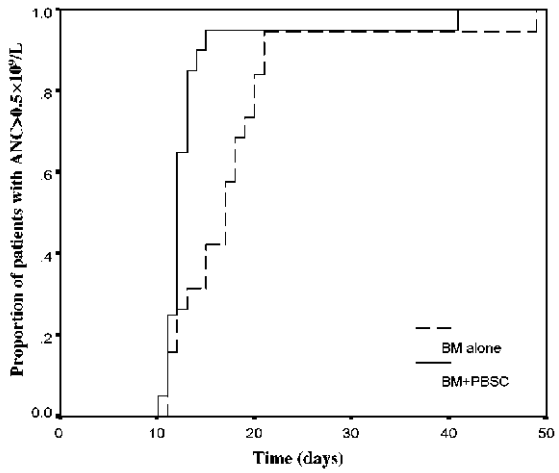

Figure 1A. Time to recovery to an ANC greater than 0.5×10^9/L shown according to the type of infused stem cells. Patients receiving BM+PBSC (solid line) recovered more rapidly than those receiving BM (dashed line) (p=0.005).

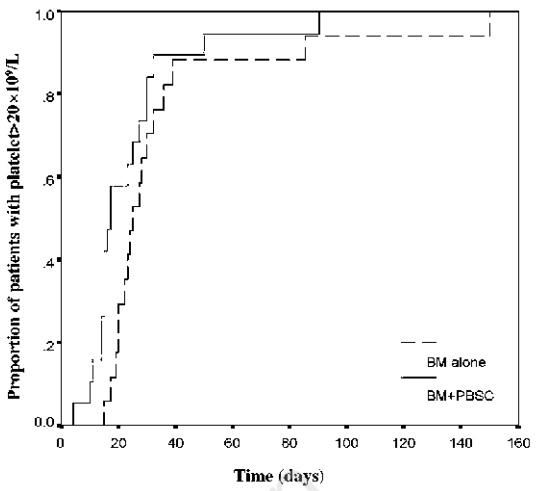

Figure 1B. Time to recovery to a platelet count greater than 20×10^9/L shown according to the type of infused stem cells. Patients receiving BM+PBSC (solid line) recovered more rapidly than those receiving BM (dashed line) (p=0.039).

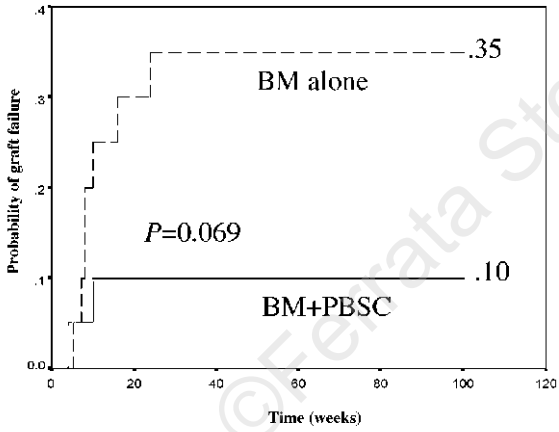

Figure 2. Effect of the type of infused stem cells on graft failure.

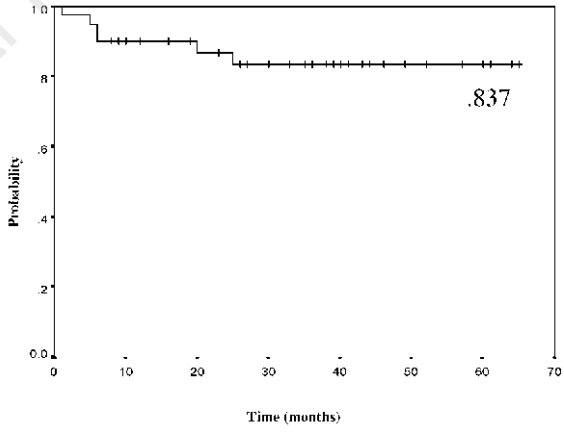

Figure 3. The probability of survival in the high-risk patients after transplantation from an HLA-identical sibling donor.

ease. In one of these 9 patients this complication was the primary cause of death. Four cases evolved from an acute GVHD (progressive type). In univariate analysis the only factor associated with a higher risk of chronic GVHD was the occurrence of acute GVHD (p=0.041). In multivariate analysis, significant factors associated with GVHD could not be found. GVHD incidence was comparable between the BM+PBSC and BM groups.

Survival

The Kaplan-Meier estimate of survival was 83.7% with a median follow-up duration of 40.5 months (range, 8-67) (Figure 3). Six of the 40 patients (15%) died: two from graft failure, two from interstitial pneumonia, one from CY-induced heart failure and one from chronic GVHD followed by pneumonia. In univariate analysis patient age (≥27.5 versus <27.5 years)

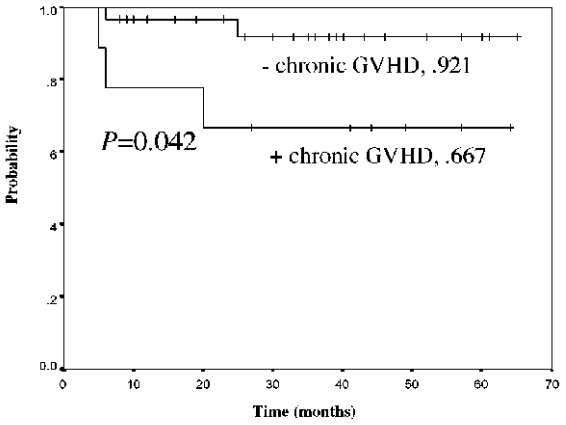

Figure 4. Survival of the high-risk patients who developed chronic GVHD, versus those who did not.

(p=0.038) and the occurrence of chronic GVHD (p=0.042) were correlated with survival. Male recipients from female donors, interval from diagnosis to transplant (≥59 versus <59 months), number of previous transfusions (≥115 versus <115 units), refractoriness to random donor platelets, ABO mismatch, the occurrence of graft failure and the type of infused stem cells (BM versus BM+PBSC) did not significantly affect survival. In multivariate analysis only chronic GVHD adversely influenced survival (p=0.042). The survival rate was 66.7% for patients who developed chronic GVHD and 92.1% for those without chronic GVHD (Figure 4).

Discussion

Transfusion is a pretransplant factor associated with graft failure because exposure to multiple transfusions sensitizes the recipient to histocompatibility antigens.[5,6] Disease duration before HSCT also has a strong impact on survival. Patients whose transplant occurred more than 3 years after diagnosis had a much lower survival rate than those whose transplant occurred within the first 3 years.[16] Early identification of donors and an expeditious transplant strategy are required for patients who have an HLA-identical sibling, before they become immunized. However, in current practice, relatively few patients (<15%) are managed without pretransplantation transfusions.[4] In Korea, many patients were not been able to receive an early transplant because the national medical insurance system could not cover BMT until comparatively late. Many patients who had a matched sibling donor, therefore, received immunosuppressive treatment prior to transplant and transfusions could not be avoided entirely before they were referred to an expert. Half of our patients were diagnosed before 1990 and they had not been able to receive irradiated, leukocyte-depleted blood products in an attempt to decrease the risk of sensitization to histocompatibility antigens.

More efficient immunosuppressive conditioning regimens and changes in transfusion support have been introduced over the last decades. Recent studies indicate that the addition of ATG to CY during the conditioning regimen leads to low graft failure.[8] However, in the absence of controlled studies, it is uncertain whether the published low graft failure rates reflect a real beneficial effect of ATG or are the result of recent modifications in transfusion practice and supportive care. Survival was not improved with addition of radiation to the conditioning regimen.[2] The addition of peripheral blood buffy coat cells is also associated with a low graft rejection rate but unfortunately it is also followed by an unacceptably high rate of chronic GVHD.[7]

Here, we report the transplant outcome of 40 consecutive patients with SAA in a single institution from 1995 to 1999. The patients had all been multi-transfused and/or had long disease duration. We used intensified pretransplant conditioning ± increasing donor cells in the infusion to overcome rejection in these high-risk patients. Although a randomized study[17] failed to show an advantage of a procarbazine/ATG-containing regimen over CY alone, synergistic immunosuppressive effects between ATG and alkylating agents, including procarbazine and CY were shown without complications associated with radiation-based regimens.[18] In our patients receiving CY+ATG+procarbazine, one case of thyroid papillary carcinoma occurred 3 years post-transplantation. Follow-up for long-term sequelae is required. A low marrow cell dose (< 3×10^8/kg of body weight) was associated with graft failure which was reduced by the infusion of larger numbers of donor hematopoietic stem cells derived from marrow supplemented by peripheral blood buffy coat cells.[19] Experience with blood stem cell allografts in aplastic anemia is very limited,[20,21] and careful study is warranted before their widespread adoption. Half of our patients received the combination of unmanipulated marrow and T-cell depleted PBSC as the stem cell source[22,23] in an attempt to increase the number of infused stem cells without increasing the risks of GVHD.

In the present study graft failure occurred in 35% of patients who received the intensified pretransplant regimen with BM alone compared to in 10% of those who were transplanted with the same conditioning and high-dose stem cells including BM + T-cell depleted PBSC. The difference was strongly suggestive (p=0.069). This distribution of patients who had graft failure may partially be explained by the fact that, in our patients, the CY-ATG-procarbazine conditioning regimen alone did not seem to provide sufficient immune suppression to overcome sensitization to histocompatibility antigens on donor cells. The infusion of a large number of stem cells could lead to a reduction of graft failure and a faster speed of engraftment. Although the two groups were comparable with respect to several factors, including age, duration of aplasia, and history of transfusion, ours was not a randomized study and maximum comparabil-

ity could not have been achieved. Although the utilization of the intensified conditioning regimen and high-dose stem cells including BM+PBSC should be studied further, in the context of heavily transfused patients they may have a beneficial effect on survival. When graft failure did develop, booster treatment with additional immune suppression and a second stem cell infusion was also successful in sustaining donor hematopoiesis again. (CK Min *et al.*, *Acta Haematologica, in press*) In fact no patient given a booster infusion of stem cells died of graft rejection. The excellent results of the second transplant are in line with those in other reports.[24,25]

In our patients who were at high risk in terms of graft failure, only chronic GVHD had a negative effect on survival after a transplant, as has been noted in many studies.[26] One patient died of chronic GVHD and 2 of interstitial pneumonia during chronic GVHD. According to the Seattle data, chronic GVHD emerged as the major cause of morbidity and mortality after transplant, and the most important risk factor for nearly all long-term complications; acute GVHD and donor buffy coat infusion, and the year of transplant significantly increased the risk of chronic GVHD.[26] In the present study the only factor associated with a high risk of chronic GVHD was the occurrence of acute GVHD. GVHD incidence was comparable between the BM+PBSC and BM groups. In our patients T-cell depletion from PBSC seemed to diminish the incidence of chronic GVHD, compared with the incidence associated with addition of buffy coat cells.

The number of blood units required to induce allosensitization and compromise the graft is unknown. According to whether 10^{27} or 20^{6} units were transfused, survival was significantly affected. Since most of our patients had received a large number of transfusions and/or had a long disease duration we chose the median levels, 115 transfusion units and 59 months, to try to define different risk groups for survival. However, differences in the number of blood units transfused and disease duration did not correlate significantly with survival in these high-risk patients who had been heavily transfused for a long time. A deleterious effect on survival of increasing age was not found in the present study. The survival at 3 years was 83.7%, which does not appear to be worse than that in other series. The *International Bone Marrow Transplant Registry* reported that the 5-year probability of survival was 68% for 696 patients who were 21 to 39 years old.[4] Despite the small numbers, our patients were all adults who had been transplanted a long time after diagnosis, and therefore had been heavily transfused.

In conclusion, this study suggests that HSCT, using an intensified conditioning regimen and a high dose of stem cells, is an effective treatment for adult patients with SAA who have an HLA-matched sibling donor and have been heavily transfused and/or have had their disease for a long time. Due to the reduced incidence of graft failure and successful second transplants, graft failure may not have a significant impact on survival, with regards to engraftment, in these high-risk patients.

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CKM contributed to the study design, collected and analyzed the data, and prepared the first draft of this manuscript. DWK had the initial idea of performing this study. JWL, CWH and WSM were responsible for the transplant procedure and reviewed the paper. CCK approved the protocol and the final version of the paper to be submitted. We would like to thank the nursing staff, residents, and fellows of the BMT unit for their dedicated care of the patients.

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Disclosures

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Potential implications for clinical practice

High doses of stem cells, using graft engineering, can be used for patients who have a long history of severe aplastic anemia and have received multiple transfusions.

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