Immune reconstitution after allogeneic stem cell transplantation: the impact of stem cell source and graft-versus-host disease

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Background and Objectives. Bone marrow (BM) and blood stem cell (BSC) allografts differ considerably with respect to their content of progenitor cells and progenitor cell subsets as well as mature lymphocytes. The aim of this prospective, randomized study was to determine whether these differences have an impact on early post-transplant immune recovery.

Design and Methods. In a prospective randomised study, we found enhanced immune recovery in recipients of BSC allografts compared to in recipients of BM allografts despite transplantation of a lower number of lymphoid progenitors, particularly B-cell progenitors. The large number of mature lymphocytes in BSC allografts is a plausible explanation for this observation. At the progenitor cell level, we found a comparable and very high proportion of progenitor cells involved in lymphopoiesis in both study groups.

Results. Patients with extensive chronic GVHD, irrespective of the allograft received, had low immunoglobulin (Ig) levels in serum, low B-cell counts in blood and low numbers of B-cell progenitors in the bone marrow. They also showed high T-cell counts, particularly $CD3^{+}CD8^{+}$ T-cell counts, which was paralleled by a high number of T-cell progenitors in the bone marrow. In patients with extensive chronic GVHD we found low natural killer (NK)-cell counts which has not been reported previously.

Interpretation and Conclusions. Early immune recovery is enhanced following BSC allografting compared with BM allografting. This is plausibly explained by the large inoculum of mature lymphocytes in BSC allografts. Following allografting, a higher proportion of the BM progenitor cell compartment is involved in lymphopoiesis than it is in healthy adults. However, B-lymphopoiesis is inhibited in patients with extensive chronic GVHD resulting in impaired B-cell recovery. These patients also seem to show impaired NK-cell recovery.

Key words: immune reconstitution, allogeneic stem cell transplantation, stem cell source, GVHD

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Blood stem cells (BSC) are being increasingly used as the source of hematopoietic stem cells for allogeneic transplantation. A randomized study conducted at our institution comparing the short-term clinical outcomes of BSC and bone marrow (BM) allografting, showed that recipients of BSC had faster neutrophil and platelet recovery than did recipients of BM.[1] Corresponding studies published to date uniformly report the same results.[2-4] Allogeneic stem cell transplantation still carries significant risks of transplant-related morbidity and mortality. The transplant-related mortality in the early post-transplant period has decreased due to improved prophylactic measures and supportive care. The faster hematologic recovery associated with BSC allografts is also assumed to have contributed to this decrease.

In the later post-transplant period, susceptibility to infections due to B- and T-lymphocyte deficiencies is a major contributor to morbidity and mortality.[5,6] This immune deficiency period may last for more than a year.[5] In fact, in a long-term follow-up study by Storek *et al.* impaired regeneration of $TREC^{+}$ $CD4^{+}$ T-lymphocytes, although clinically insignificant, seemed to extend beyond 20 years post-transplant.[7]

Unmanipulated BSC allografts contain several times more lymphocytes than do BM allografts.[8-10] Although the recipients may benefit in the short term from the increased number of B- and T-cells, recipients of both unmanipulated and T-cell depleted allografts predominantly reconstitute their B- and T-cell repertoire through donor stem cell-derived lymphopoiesis after transplantation.[11] BSC

Table 1. Patients' characteristics.

	Blodd stem cell n=25	*Bone marrow n=22*
Median age at tx (range)	36 (16-53)*	46 (27-56)*
Gender (male/female)	16/9	10/12
Diagnosis		
Acute lymphoblastic leukemia	1	4
Acute myeloid leukemia	11	7
Chronic myeloid leukemia	11	11
Primary myelofibrosis	2	0
Donor-recipient histocompatibility		
HLA-identical	21	21
One HLA-A, B or DR antigen mismatch	4	1
Acute GVHD	10	6
Grade 1-2	6	4
Grade 2-4	4	2
Chronic GVHD	15	11
Limited	5	7
Extensive	10	4

*Tx: transplantation; GVHD: graft-vs-host disease. *Age at tx. is significantly different between the groups (p=0.007).*

and BM grafts also differ in terms of number and composition of CD34+ progenitor cells. BSC grafts contain 2-4 times more CD34+ cells than do BM grafts,[2] and immunophenotypic assessment has revealed a substantial increase in the number of myeloid progenitors at the expense of lymphoid progenitors in BSC grafts as compared with BM grafts.[12]

The present study was conducted to address whether such differences in the composition of the allografts result in differences in the immune reconstitution, particularly B-cell reconstitution within the first year after allografting. Forty-seven patients with hematologic malignancies who were candidates for allogeneic stem cell transplantation were randomly assigned to receive BSC or BM grafts. Immunoglobulin (Ig) levels in serum were assessed at 3, 6, 9 and 12 months, and lymphocyte subsets in blood and CD34+ progenitor cells in bone marrow were enumerated at 3, 6 and 12 months post-transplant. The data were analyzed according to the type of allograft received and the absence or presence of extensive chronic graft-versus-host disease (GVHD).

Design and Methods

Patients and controls

Between June 1994 and February 1999, 61 patients with hematologic malignancies were included in a randomized single center study comparing short-term clinical outcomes in patients receiving BSC or BM allografts.[1] Of these, 57 patients were included in the immune reconstitution study. Written informed consent was obtained using protocols approved by the Regional Medical Research Ethics Committee, Southern Norway (REC II). Four patients were not included due to very early death. Of the 57 patients included in the study, 10 patients were excluded from data analysis because data of interest could not be procured due to early relapse, Epstein Barr virus-associated lymphoma or treatment-related death within 3 months of transplantation. The characteristics of the 47 patients who finally constituted the study population are provided in Table 1. There is a statistically significant difference in age between the groups, but the groups are comparable with respect to the other parameters. An age difference was not present in the original cohort of 61 patients, and it is accounted for by the loss of 14 of the 61 patients for the data analysis of this study. Institutional reference values for lymphocyte subsets and subsets of CD34+ progenitor cells had been established in healthy adults.[12,13]

Harvest procedures

BSC were mobilized with filgrastim (r-metHuG-CSF, Hoffman-La Roche, Basel, Switzerland), and a minimum of 2×10^6 CD34+ cells/kg recipient weight was collected by leukapheresis. BM allografts were procured by aspiration from the posterior iliac crests, and a minimum of 2×10^8 nucleated cells/kg recipient weight was collected. The harvest procedures are described in detail elsewhere.[14]

Conditioning and GVHD prophylaxis/treatment

All patients were conditioned with busulphan (16 mg/kg) and cyclophosphamide (120 mg/kg). Patients with acute lymphoblastic leukemia and acute myeloid leukemia (M4/M5) received intrathecal methotrexate (12 mg), two doses as part of the conditioning regimen and four doses post-transplant. Methotrexate and cyclosporine A were given to prevent GVHD. Acute GVHD was graded continuously according to the Glucksberg criteria[15] and was treated with intravenous methylprednisolone followed by oral prednisolone (grade II-IV).[1] Chronic GVHD was classified as described by Shulman *et al.*[16] None of the patients received Ig supplementation during the study period.

Cell preparation and staining

Blood and bone marrow samples were collected 3, 6 and 12 months after transplantation. Unfractionated blood was labeled with the following monoclonal antibodies: fluorescein isothiocyanate (FITC)-conjugated anti-CD3, anti-CD5, anti-CD45RA, anti-

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CD45RO and anti-HLA-DR, and phycoerythrin (PE)-conjugated anti-CD4, anti-CD8, anti-CD16, anti-CD19 and anti-CD56 (all purchased from ImmunoQuality Products, Groningen, The Netherlands). FACS Lysing Solution (Becton Dickinson Immunocytometry Systems (BDIS, San José, CA, USA) was used to lyse erythrocytes after staining. Bone marrow was collected into preservative-free heparin (Monoparin, CP Pharmaceuticals Ltd., Wrexham, UK). Mononuclear cells were prepared by density gradient centrifugation (Lymphoprep, 1.077 g/L, Nycomed Pharma, Oslo, Norway) and labeled with the following monoclonal antibodies: FITC-conjugated anti-CD2, anti-CD7, anti-CD10, anti-CD14, anti-CD15, anti-CD20, anti-CD34, anti-CD42a, anti-CD61 or anti-HLA-DR and PE-conjugated anti-CD13, anti-CD33, anti-CD34 or anti-CD38 (all antibodies purchased from BDIS). Finally, the stained cells from both blood and bone marrow were resuspended in 1% paraformaldehyde.

Flow cytometry

Flow cytometry was performed on a FACScan (BDIS) or a FACSort (BDIS) equipped with a 15mW air-cooled argon laser tuned at 488 nm. A gate identifying lymphocytes in blood was drawn in a dual parameter cytogram of forward scatter versus orthogonal light scatter. Cellular events (>5,000 in all cases and time points) satisfying this gate were acquired, stored in list mode files and analyzed for two-color fluorescence. $CD34^+$ bone marrow cells were identified in a cytogram of orthogonal light scatter versus fluorescence, as previously described.[12] Cellular events (>1,000 in all cases and time points) satisfying this gate were acquired, stored in list mode files and analyzed for two-color fluorescence. Data acquisition and analysis were performed either by the Lysis II (BDIS) or CellQuest (BDIS) research software.

Immunoglobulins

Serum levels of IgA, IgG and IgM were quantified by nephelometry (Dade Behring, Liederbach, Germany) 3, 6, 9 and 12 months post-transplant.

Statistics

Non-parametric analyses were used throughout. Data are presented as median (range) unless otherwise stated. Groups of patients were compared using a two-sided Mann-Whitney's U test. Correlation analyses were performed using Spearman's rank test. p-values less than 0.05 are considered statistically significant. Calculations were performed with the Statistica software (Statsoft, Tulsa, OK, USA).

Results

Graft characteristics

Compared with BM recipients, BSC recipients received significantly higher numbers of total nucleated cells, $CD34^+$ cells, $CD3^+$ T cells, $CD3^+CD4^+$ T cells, $CD3^+CD8^+$ T cells, CD3-CD16/$CD56^+$ NK cells and $CD20^+$ B cells (Table 2). The number of $CD34^+$ cells was 1.4 times higher and the number of $CD3^+$ cells 4.9 times higher in the BSC grafts. We have previously established reference values for the different subsets of $CD34^+$ cells in BM and BSC grafts procured from healthy adults.[12,13] Based on these reference values we calculated the number of different progenitor cell subsets delivered by the respective allografts (Table 2). BSC recipients received a higher number of immature and myeloid progenitors and a lower number of B-lymphoid progenitors than did BM recipients (Table 2). The number of $CD34^+CD2^+$, but not $CD34^+CD7^+$ progenitor cells was higher in the BM grafts than in the BSC grafts.

Lymphocyte reconstitution

At 3 months, the total lymphocyte count was higher in the patients allografted with BSC than in those allografted with BM (median 1140 and 887×10^6/L, respectively). This was also the case for all lymphocyte subsets investigated: $CD19^+$ B-cells (median 50.3 and 16.5×10^6/L, p=0.02), $CD5^+CD19^+$ B-cells cells (median 27.7 and 6.9×10^6/L, p=0.018), $CD3^+$ T cells (median 956.1 and 410.0×10^6/L, p=ns), $CD3^+CD4^+$ T-cells (median 328.1 and 169.5×10^6/L, p=0.027), $CD4^+$ $CD45RA^+$ T-cells (median 92.3 and 17.7×10^6/L, p=0.032), $CD4^+$ $CD45RO^+$ T-cells (median 184.8 and 165.6×10^6/L, p=ns), $CD4^+$ HLA-DR^+ T-cells (median 99.0 and 59.5×10^6/L, p=ns), $CD3^+CD8^+$ T-cells (median 760.7 and 436.8×10^6/L, p=ns), $CD8^+$HLA-DR^+ T-cells (median 202.4 and 152.9×10^6/L, p=ns), $CD3^+CD16^+/56^+$ T cells (median 100.2 and 19.5×10^6/L, p=0.005) and CD3-$CD16^+/56^+$ NK cells (median 110.5 and 107.5×10^6/L, p=ns) (Figure 1).

The total lymphocyte count, $CD3^+$ T-cell count and $CD3^+CD4^+$ T-cell count remained higher in the patients allografted with BSC throughout the study period (Figure 1). However, these differences were no longer statistically significant at 6 and 12 months. No difference in serum Ig levels was detected at 3, 6, 9 or 12 months between patients in the two study groups, and median Ig levels were within the normal range at all time points (Table 3).

$CD34^+$ bone marrow cells

$CD34^+$ cells constituted approximately 2% of mononuclear bone marrow cells in both study groups at 3, 6 and 12 months after transplantation.

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Table 2. **Graft characteristics. Median number of cells (range) per recipient body weight.**

	Blood Stem Cells Median (range)/kg/bw	Bone Marrow Median (range)/kg/bw	p
Nucleated cells×10^8	9.35 (3. 9-15.2) n=24	3.30 (2.0-8.2) n=22	<0.001
$CD34^+$ cells (total)×10^6	3.1 (2.1-7.7) n=25	2.15 (1.0-4.7) n=13	0.01
T-cell progenitors			
$CD34^+CD2^+$ cells×10^6	0.06 (0.04-0.2)	0.09 (0.04-0.2)	0.03
$CD34^+CD7^+$ cells×10^6	0.08 (0.05-0.2)	0.07 (0.03-0.1)	ns
B-cell progenitors			
$CD34^+CD10^+$ cells×10^6	0.09 (0.06-0.2)	0.6 (0.3-1.3)	<0.001
$CD34^+CD19^+$ cells×10^6	0.05 (0.04-0.1)	0.4 (0.2-0.9)	<0.001
$CD34^+CD20^+$ cells×10^6	0.03 (0.02-0.08)	0.09 (0.04-0.2)	<0.001
Myeloid progenitors			
$CD34^+$ $CD13^+$ cells×10^6	2.7 (1.8-6.7)	1.3 (0.6-2.8)	<0.001
Immature progenitors			
$CD34^+$HLA-DR- cells×10^6	0.2 (0.1-0.4)	0.08 (0.04-0.2)	<0.001
$CD34^+$CD38- cells×10^6	0.1 (0.07-0.2)	0.05 (0.02-0.1)	<0.001
$CD3^+$ cells×10^8	2.34 (0.6-4.6)	0.48 (0.3-0.9)	<0.001
$CD3^+CD4^+$ cells×10^8	1.20 (0.5-2.6)	0.27 (0.2-0.5)	<0.001
$CD3^+CD8^+$ cells×10^8	0.98 (0.2-2.2)	0.19 (0.09-0.4)	<0.001
CD3-$CD16^+/56^+$×10^8	0.23 (0.02-0.6)	0.056 (0.03-0.1)	<0.001
$CD20^+$ cells×10^8	0.54 (0.1-1.5)	0.16 (0.07-0.4)	<0.001

The characteristics of one of the BSC grafts and twelve of the BM grafts were not complete. ns: not statistically significant.

No significant differences between the two groups were found with respect to $CD34^+$ cell subsets (Table 4). However, compared to $CD34^+$ bone marrow cells from healthy individuals at steady state the immunophenotypic profile of $CD34^+$ bone marrow cells during the first year after transplantation was strikingly different (Table 4). The proportion of lymphoid progenitors, in particular B-cell progenitors, was increased at the expense of myeloid progenitors after transplantation. This was most conspicuous during the first 6 months and tended to decline with time, but was still present at 12 months.

Chronic GVHD

To assess the effect of chronic GVHD on immune reconstitution we compared patients with extensive chronic GVHD to patients with no or limited chronic GVHD. Regardless of which type of allograft was received, the $CD19^+$ B-cell counts tended to be lower (p=0.5 at 3 months, p=0.018 at 6 months and p=0.5 at 12 months) and the CD3+ T-cell and $CD3^+CD8^+$ T-cell counts tended to be higher (p=0.24 and 0.31 at 3 months, respectively, p=0.72 and 0.85 at 6 months, respectively, p=0.75 and 0.50 at 12 months, respectively) in the patients with extensive chronic GVHD throughout the study period (Figure 2). We also

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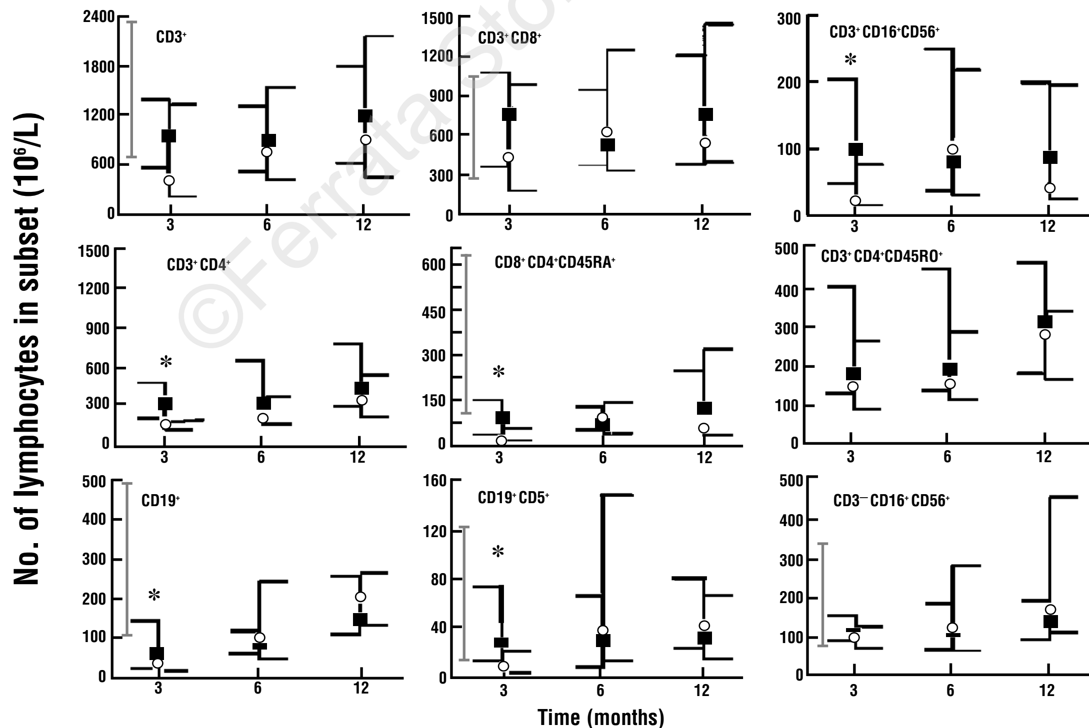

Figure 1. **Median values of various lymphocyte subsets in peripheral blood as indicated, 3, 6, and 12 months following allografting with BSC (filled squares, whole lines, left-sided whiskers) and BM (open circles, dotted lines, right-sided whiskers). Lower and upper whiskers indicate the 25th and 75th percentiles, respectively. Ranges for normal reference values are indicated by vertical gray line where applicable. Statistically significant differences are marked by an asterix.**

found that the CD3⁻CD16⁺/56⁺ NK-cell counts were lower in the patients with extensive chronic GVHD compared to in the patients with no or limited chronic GVHD. This difference was statistically significant at all time points (p=0.019, p=0.021 and p=0.031, respectively). However, we did not find that the CD4⁺ (Figure 2) or CD3⁺CD16/56⁺ T-cell counts were influenced by GVHD.

Ig levels in patients with extensive chronic GVHD were lower than in patients with no or limited chronic GVHD (Table 3). This was true for IgA as well as for IgG and IgM, but the difference was only found to be statistically significant for IgA (p<0.02 at all time points). At the progenitor cell level, we found differences with respect to B-cell, T-cell and myeloid progenitors. The proportion of B-cell progenitors was reduced and the proportion of myeloid progenitors was increased in the patients with extensive chronic GVHD. This was already evident at 3 months, but statistically significant differences between the study groups were only found at 6 and 12 months (Table 5). In contrast to B-cell progenitors, the proportion of T-cell progenitors was increased in the patients with extensive GVHD (Table 5). However, this was only found to be statistically significant for CD34⁺CD7⁺ progenitor cells at 6 months.

We performed correlation analyses to study the possible association between serum Ig levels, B-cell counts in the peripheral blood and B-cell progenitors in the bone marrow in patients with extensive GVHD. We found associations between IgA levels and B-cell counts (rs=0.82, p<0.05), IgG levels and B-cell counts (rs=0.79, p<0.05) and IgG levels and B-cell progenitors (rs=0.80, p<0.05).

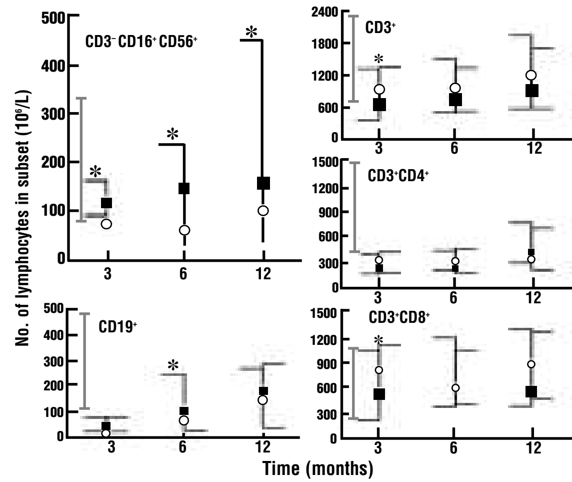

Figure 2. Median values of various lymphocyte subsets in peripheral blood as indicated, 3, 6, and 12 months following allografting in patients with no or limited GVHD (filled squares, whole lines, left-sided whiskers) and patients with extensive chronic GVHD (open circles, dotted lines, right-sided whiskers). Lower and upper whiskers indicate the 25th and 75th percentiles, respectively. Ranges for normal reference values are indicated by a vertical gray line. Statistically significant differences are marked by an asterix.

Discussion

We performed a prospective randomized study examining immune reconstitution following allogeneic stem cell transplantation with BM or BSC. Lymphocyte counts were higher in BSC recipients, and these were statistically significant for the following lymphocyte subsets: CD19⁺ B cells, CD19⁺CD5⁺ B cells, CD3⁺CD4⁺ T cells, CD4⁺CD45RA⁺ T cells and

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Table 3. Immunoglobulin levels (median and range) within the first year of allogeneic stem cell transplantation.

	3 months			*6 months*			*9 months*			*12 months*		
	IgG g/L	*IgA g/L*	*IgM g/L*	*IgG g/L*	*IgA g/L*	*IgM g/L*	*IgG g/L*	*IgA g/L*	*IgM g/L*	*IgG g/L*	*IgA g/L*	*IgM g/L*
BM	10,7	1.28	0.81	11.1	1.27	0.93	10.7	1.55	1.5	10.95	1.57	1.38
	(6.2-15)	(0.44-2.63)	(0.32-2.09)	(7.2-14.7)	(0.38-2.76)	(0.34-1.77)	(7.7-15.6)	(0.51-3.3)	(0.54-3.89)	(7.8-19.1)	(0.5-3.38)	(0.6-4.82)
BSC	9.15	1.2	0.72	9.25	1,2	0,96	11.7	1,37	1.28	11.1	1.34	0.83
	(6.5-15)	(0.28-3.7)	(0.23-2.12)	(6.3-19.9)	(0.19-3.45)	(0.21-1.67)	(5.9-35.2)	(0.25-3.54)	(0.38-3.2)	(5.33-36.9)	(0.28-2.9)	(0.43-1.7)

Impact of GVHD on immunoglobulin levels (median and range) within the first year of allogeneic stem cell transplantation

	3 months			*6 months*			*9 months*			*12 months*		
	IgG g/L	*IgA g/L*	*IgM g/L*	*IgG g/L*	*IgA g/L*	*IgM g/L*	*IgG g/L*	*IgA g/L*	*IgM g/L*	*IgG g/L*	*IgA g/L*	*IgM g/L*
No GVHD	10.8	1.28	0.95	11.5	1.45	1.05	11.7	1.57	1.33	11.1	1.57	1.15
	6.6-14.6	0.59-3.7	0.32-2.12	7.0-14.7	0.43-3.45	0.44-1.77	7.0-15.6	0.45-3.54	0.47-3.46	7.8-19.1	0.48-3.38	0.52-4.75
GVHD	8.3	0.69	0.46	8.5	0.72	0.84	9.0	0.88	1.78	9.0	0.78	1.04
	6.2-15	0.28-2.2	0.23-1.65	6.3-19.9	0.19-1.98	0.21-1.59	5.9-35.2	0.25-1.99	0.38-3.89	5.33-36.9	0.28-1.73	0.43-4.82

Table 4. Progenitor cell subsets in bone marrow during reconstitution.

	Progenitor cell subsets(% of CD34+ cells, median and quartiles)						
	3 months		6 months		12 months		Normal controls
	BSC	BM	BSC	BM	BSC	BM	Mean±2SD
$CD34^+$ cells*	1.95	1.46	2.3	2.4	1.9	1.4	2.8±1.2
T-cell progenitors							
$CD34^+CD2^+$	8.0	7.0	8.3	6.5	6.2	8.7	5.6±2.7
	4.5-9.2	5.9-9.9	5.0-13.0	3.9-13.0	3.8-8.7	4.7-10.1	
$CD34^+CD7^+$	8.3	7.0	9.5	6.0	6.1	6.5	4.9±2.3
	4.1-11.6	5.1-18.9	5.5-16.9	3.1-14.7	4.0-9.6	5.2-11.8	
B-cell progenitors							
$CD34^+CD10^+$	40.8	49.3	40.6	42.4	23.4	36.1	21.4±10.2
	22.4-48.4	32.5-55.3	30.2-55.9	29.8-59.3	15.3-28.7	18.3-47.5	
$CD34^+CD20^+$	11.0	15.6	12.8	12.8	6.9	14.2	1.0±0.2
	9.7-16.5	11.0-19.3	6.4-16.7	8.6-20.0	6.5-8.8	9-15.2	
Myeloid progenitors							
$CD34^+CD13^+$	56.0	54.7	56.9	59.5	75.2	64.4	82.1±14.2
	50.5-86.0	40.5-62.7	39.0-67.9	48.1-72.3	58.8-82.6	45.8-70.3	
$CD34^+CD33^+$	54.7	43.2	52.4	44.8	65.4	53.3	71.5±16.3
	40.1-65.3	38.9-56.7	35.4-65.0	36.9-70.7	55.5-87.1	42.9-65.3	
Thrombocyte progenitors							
$CD34^+CD42a^+$	4.1	4.3	4.1	5.4	6.2	5.4	4.2±2.8
	2.2-6.0	2.5-5.9	2.8-9.2	3.1-8.6	2.9-10.7	3.3-8.9	
$CD34^+CD61^+$	5.6	6.0	5.5	8.5	10.3	8.0	6.3±4.2
	3.0-8.4	3.9-9.8	3.8-13.1	4.4-16.1	5.2-21.5	5.1-12.3	
Immature progenitors							
$CD34^+HLA\text{-}DR^-$	1.7	1.9	1.7	2.0	2.0	1.7	2.3±1.8
	1.2-2.9	0.9-4.0	0.9-3.4	0.6-3.7	1.4-3.0	0.7-3.6	
$CD34^+CD38^-$	0.7	0.6	0.4	0.6	0.3	0.6	0.5±0.8
	0.2-1.5	0.5-2.4	0.1-1.4	0.2-1.8	0.2-1.2	0.2-4.3	

**% of mononuclear bone marrow cells.*

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Table 5. Progenitor cell subsets in bone marrow during immune reconstitution and the impact of GVHD.

	Progenitor cell subsets (% of $CD34^+$ cells, median)					
	3 months		6 months		12 months	
	N/L	Ext.	N/L	Ext.	N/L	Ext.
T-cell progenitors						
$CD34^+CD2^+$	6.9	8.3	5.6	10.2	6.1	7.4
$CD34^+CD7^+$	6.2	12.3	6.0	16.9[1]	5.6	7.6
B-cell progenitors						
$CD34^+CD10^+$	44.8	38.5	45.6	29.0[2]	34.4	15.3[4]
$CD34^+CD20^+$	14.1	12.1	14.8	6.7[3]	11.5	6.8[5]
Myeloid progenitors						
$CD34^+CD13^+$	54.9	40.5	49.6	65.3	65.8	73.9
$CD34^+CD33^+$	43.6	50.8	43.6	68.9[6]	55.0	65.4

N/L: no/limited; Ext.: extensive. [1]p:0.037; [2]p:0.003; [3]p:0.007; [4]p:0.002; [5]p:0.012; [6]p:0.012.

$CD3^+CD16/56^+$ T cells. The proportion of B-cell progenitors in the bone marrow was significantly higher in both BM and BSC recipients than in healthy adults. This increase in B-cell progenitors was compromised in the patients with extensive chronic GVHD in whom we found the proportion of T-cell progenitors to be increased. These patients also had low NK-cell counts compared to patients with no or limited chronic GVHD. When we planned this study we hypothesized that the reduced number of lymphoid progenitors in BSC allografts might impair early immune recovery, particularly B-cell recovery. However, during the course of our study results of other small, non-randomized studies indicated that the large number of lymphocytes infused to BSC recipients resulted in higher lymphocyte counts, particularly T-cell counts, after transplantation.[9,10] Higher blood lymphocyte counts during the first year after transplantation in BSC allograft recipients has recently been confirmed in a prospective non-randomized

study.[17] The results of the present study fit those of Storek *et al*.[17] very well.

The major difference between the studies is that we found statistically significant higher lymphocyte subset counts only at 3 months, whereas Storek *et al*. reported statistically significantly higher T-cell counts throughout the first year. Higher T-cell counts in recipients of BSC allografts have been attributed to the large inoculum of T cells, because T cells, especially the naïve phenotype, are long-lived.[18-20] In the first year after transplantation most T cells originate from T cells infused with the allograft.[21-24] In the study by Storek *et al*.[17] the BSC grafts were 8-fold enriched for $CD8^+$ T-cells and 12-fold enriched for $CD4^+$ T cells compared to BM grafts, whereas our BSC grafts were 4-fold enriched for these T-cell subsets. Along with a higher number of patients included in the study by Storek *et al*., this difference in graft characteristics provides a reasonable explanation for the difference between the two studies with respect to T-cell counts within the first year of transplantation.

We also studied immune recovery at the progenitor cell level and found no difference between the study groups. However, we show that a higher proportion of $CD34^+$ cells is engaged in lymphoid differentiation after transplantation than in healthy adults. We are prone to consider the number of lymphoid progenitors in the bone marrow as an index of lymphopoiesis. This notion is supported by the observation that a higher number of $CD34^+$ cells co-expresses CD10, CD19 and CD20 in children than in adults[25] and by our recent report that the number of lymphoid progenitors is increased in patients with severe human immunodeficiency virus-infection and decreases during highly active anti-retroviral therapy as lymphocyte counts recover.[13]

B-cell deficiency is a well-recognized feature of chronic GVHD.[5,26,27] This has been attributed to an inhibition of B-lymphopoiesis by GVHD and/or its treatment.[26,27] We analyzed our data with respect to progenitor cell subsets in the presence or absence of severe chronic GVHD and found a low number of B-cell progenitors in the patients suffering from severe chronic GVHD. The proportion of B-cell progenitors decreased with time. Low numbers of B-cell progenitors correlated with low B-cell counts in blood and low Ig levels in serum, in particular IgA levels. This finding provides support to the notion that B-lymphopoiesis is inhibited in chronic GVHD. On the other hand, T-cell counts, especially $CD8^+$

T-cell counts, tended to be higher in patients with severe chronic GVHD compared with those with no or limited GVHD. Our data also indicate that high T-cell counts in patients with extensive chronic GVHD are linked to an increased number of T-cell progenitors in the bone marrow. However, our data cannot be considered as evidence for increased T-cell lymphopoiesis in the thymus in these patients. Furthermore, we found low NK-cell counts in blood in the patients with severe chronic GVHD. Two distinct subsets of human NK cells have been identified according to cell surface expression of CD16 and CD56. A $CD16^{bright}CD56^{dim}$ subset and a $CD16^{dim/neg}CD56^{bright}$ subset, which accounts for 90% and 10% of human NK cells respectively.[28] The design of our flow cytometry protocol did not allow us to distinguish between these two subsets of NK cells. To our knowledge, this has not been highlighted in previous reports on immune reconstitution following allogeneic stem cell transplantation. On the contrary, rapid NK-cell recovery has usually been reported following allografting. However, Shenoy *et al*.[29] reported on low NK-cell counts and impaired NK-cell function within the first year following BSC allografting. Extensive chronic GVHD was present in 72% of their patients which indicates that their findings are in line with ours.

In conclusion, we confirm previous reports that early immune recovery is enhanced following BSC allografting compared with BM allografting. This is provided by the large inoculum of mature lymphocytes with the BSC allografts. Following allografting, a higher proportion of the bone marrow progenitor cell compartment is involved in lymphopoiesis than it is in healthy adults. However, B-lymphopoiesis is inhibited in patients with extensive chronic GVHD resulting in impaired B-cell recovery. These patients also seem to show impaired NK-cell recovery. The mechanism of impaired NK-cell recovery was not determined because the issue of NK-cell progenitors was not addressed by our study.

DH, TE and GET were all heavily involved in the planning and design of the study. IWA, SS and DK were the main contributors regarding analysis of data, while all co-authors were involved in interpretation of data. IWA and GET were responsible for drafting the article, while all co-authors revosed the manuscript and gave their final approval. The authors are indebted to Anne-Grethe Fjellman for her great efforts to organize the procurement of blood and bone marrow samples. The authors declare that they have no potential conflicts of interest.

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