Hodgkin's disease

High-dose therapy in patients with Hodgkin's disease: the use of selected CD34+ cells is as safe as unmanipulated peripheral blood progenitor cells

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Summary:

Register data suggest that patients with Hodgkin's disease (HD) given high-dose therapy (HDT) with peripheral blood progenitor cells (PBPC) have a less favourable prognosis as compared to those given bone marrow as stem cell support. Since this can be due to infusion of tumour cells contaminating the PBPC grafts, we initiated a feasibility study in which PBPC grafts from HD patients were purged by CD34+ cell enrichment. Controversy exists about whether the use of CD34+ enriched stem cells leads to a delayed haematological and immune reconstitution. We compared these parameters, including risk of infections and clinical outcome after HDT, in patients with HD given either selected CD34+ cells or unmanipulated PBPC as stem cell support. From October 1994 to May 2000, 40 HD patients with primary refractory disease or relapse were treated with HDT and supported with either selected CD34+ cells ($n = 21$) or unmanipulated PBPC ($n = 19$) as stem cell support. All patients had chemosensitive disease at the time of transplantation. A median of 5.8 (range 2.7–20.0) *vs* 4.5 (range 2.3–17.6) $\times 10^6$ CD34+ cells per kilo were reinfused in the CD34+ group and PBPC group, respectively. No difference was observed between the two groups with regard to time to haematological engraftment, reconstitution of B cells, CD56+ cells and T cells at 3 and 12 months and infectious episodes after HDT. Two (5%) treatment-related deaths, one in each group, were observed. The overall survival at 4 years was 86% for the CD34+ group and 74% for the PBPC group with a median follow-up of 37 months (range 1–61) and 46 months (range 4–82), respectively ($P = 0.9$). The results of this study demonstrate that the use of CD34+ cells is safe and has no adverse effects either with respect to haematological, immune reconstitution or to infections after HDT. *Bone Marrow Transplantation* (2001) **28,** 849–857.

Peripheral blood progenitor cells (PBPC) have replaced bone marrow (BM) as the preferred stem cell source, since the use of PBPC results in a faster haematological recovery after high-dose therapy (HDT).[1] However, studies have shown that tumour cells from different types of malignancies such as NHL, breast cancer and myeloma, are mobilised together with the CD34+ cells into the peripheral blood.[2–5] Since previous reports indicate that infusion of tumour cells contaminating the bone marrow grafts contributes to relapse after HDT,[6,7] several methods have been developed for purging of tumour cells from the grafts.[8]

HDT is increasingly used for patients with relapsed Hodgkin's disease (HD). Register data indicate that patients with HD supported with PBPC have a poorer outcome as compared to those given BM.[9] Whether this is related to infusion of contaminating tumour cells in the graft is at present not known. However, it has been shown that patients with HD have circulating clonogenic tumour cells, expressing the Hodgkin-associated surface antigen CD30.[10] Furthermore, we like others, have recently reported that patients with HD have a high number of atypical CD30+ cells in PBPC.[11–13] Therefore, removal of such unwanted cells prior to infusion might be of clinical benefit.[14,15]

The positive selection of CD34+ cells from PBPC has been used as the purging method in different types of cancer and gives a 3–4 log tumour cell removal without any significant negative influence on the haematological recovery after HDT.[16,17] Since the Hodgkin cells do not express the CD34 antigen and other purging methods for HD are not clinically available, we purified the PBPC products by enrichment of the CD34+ cells. Recently, we reported that the atypical CD30+ cells were efficiently removed by CD34+ cell enrichment and that the use of such grafts gave a fast and sustained engraftment.[11,12]

A delayed lymphoid reconstitution of both B cells and T cells and an increased risk of infections have been observed after the use of selected CD34+ cells.[18–21] Other reports, however, have not detected any difference between selected CD34+ cells and unmanipulated PBPC.[3,22–24] Fur-

thermore, it is known that HD patients have an immune dysfunction and an impaired immune response.[25]

Here, we present the results of haematological and immune reconstitution, the risk of infection and survival with the use of selected CD34+ cells and compare the data with another group of HD patients who received unmanipulated PBPC. Our results show that the use of selected CD34+ cells ($\geqslant 2.7 \times 10^6$ cells/kg) as stem cell support in HD patients is safe and bears no adverse effect either on haematological or on immune reconstitution after HDT.

Patients and methods

Patients

Between October 1994 and May 2000, 44 consecutive patients with HD were eligible for HDT and given autologous stem cell support at The Norwegian Radium Hospital. For most of that time, our hospital was the single referral centre in Norway for HDT in HD. Four patients were excluded from the study due to low numbers of progenitor cells in the PBPC ($<2 \times 10^6$ CD34+ cells/kg) and required additional bone marrow harvest. Therefore, a total of 40 patients were given either unmanipulated PBPC ($n = 19$) (PBPC group) or selected CD34+ cells ($n = 21$) (CD34+ group).

Initially, the majority of the HD patients were reconstituted with unmanipulated PBPC. However, from May 1997 a protocol of CD34+ selection was initiated, and CD34+ cell selection of the PBPC was performed in all HD patients achieving at least $6-10 \times 10^6$ CD34+ cells/kg in the leukapheresis product. Three patients were unable to mobilise this number of CD34+ cells and were reconstituted with the total amount of 2.2, 2.3 and 4.0 CD34+ cells/kg of unmanipulated PBPC. One additional patient received unmanipulated PBPC due to technical problems with the CD34+ cell procedure in the laboratory.

The clinical inclusion criteria in the study were: (1) patients who failed to achieve a CR after first-line combination chemotherapy and whose disease was not presumed to be controlled by local radiotherapy (primary refractory disease); (2) patients with relapse within 2 years after standard first-line chemotherapy; and (3) patients with second or later relapse. Patients aged less than 65 years, with chemosensitive disease reaching at least a PR after second- or third-line combination chemotherapy, WHO performance status $\leqslant 1$ and adequate cardiac, hepatic and renal function were included. All tumours were reviewed and classified according to the Revised European American Classification of Lymphoid Neoplasms (REAL).[26] The protocol was approved by the local ethics committee. Informed consent for participation in the study was obtained from all patients. The initial characteristics of the patients are listed in Table 1. As can be shown, no significant difference in the clinical characteristics of the two HD groups except for age was observed.

First-line and salvage therapy before HDT

Prior to HDT most of the patients were heavily treated with different combination chemotherapy regimens and 23 of the patients received additional radiotherapy (Table 1). From

Table 1 Patient characteristics and treatment before HDT

Number of patients	PBPC n = 19	CD34+ n = 21
Male/Female	16/3	12/9
Age at diagnosis, median (range)	32 (13–61)	22 (9–52)
Age at HDT, median (range)	35 (14–63)	25 (20–54)
Stage at diagnosis		
I	0	1
II	5	8
III	5	9
IV	8	3
Unknown	1	0
B symptoms	10	7
Bulky $\geqslant 6$ cm	7	12
LDH		
$\geqslant$normal	3	7
$<$normal	10	10
Unknown	6	3
Bone marrow involvement at diagnosis	4	1
Histology		
Nodular sclerosis	7	14
Mixed cellularity	7	4
Nodular lymphocyte-predominant	1	0
HD–not otherwise specified	4	3
First-line treatment, chemotherapy		
ABOD	10	12
EBVP	3	5
LVPP/ABOD or MVPP/Adriamycin	2	1
Other chemotherapy[a]	4	3
Radiotherapy		
Mantle field/inverted Y-field	5	7
Involved field	6	5
Induction treatment before HDT		
MIME[b]	12	19
Others + MIME	4	1
Dexa-BEAM[c]	3	1
No. of chemotherapy regimens before HDT		
2	14	19
$\geqslant 3$	5	2
Months from diagnosis to HDT, median (range)	20 (9–101)	20 (10–251)
Status at transplantation		
1 CR	2	0
1 PR	3	4
2/3 CR	8	5
2 PR	5	11
3/4 PR	1	1
Tumour mass at transplantation		
In CR	10	5
<5 cm	6	12
5–10 cm	2	3
>10 cm	0	1
bone	1	0

ABOD = adriamycin, bleomycin, vincristine, dacarbazine; EBVP = epirubicin, bleomycin, vinblastin, prednisolone; LVPP = chlorambucil, vinblastin, procarbazine, prednisolone; MVPP = mustine, vinblastine, procarbazine, prednisolone.
[a]Other chemotherapy = patients received treatment for non-Hodgkin lymphoma, later review of histology showed Hodgkin's disease.
[b]MIME.[27]
[c]Dexa-BEAM.[28]

the time the patients were scheduled for HDT, they were given salvage chemotherapy with either MIME[27] (36 patients) or Dexa-BEAM[28] (four patients) in order to reduce tumour volume and to confirm tumour chemosensitivity.

PBPC collection and CD34+ cell enrichment

As described previously,[27] the PBPC mobilisation regimen consisted of either MIME and G-CSF (36 patients) or Dexa-BEAM plus G-CSF (four patients). When $>20 \times 10^3$ CD34+ cells per ml were detected in the blood, leukapheresis was started using a CS 3000 Fenwall Cell Separator (Baxter Deerfield, IL, USA). Ten litres of blood were processed for 1 to 3 consecutive days. During the leukapheresis 300 ml plasma was collected. Before disconnecting the bag containing the PBPC from the cell separator, the platelets were removed by a specially designed soft spinning program in the CS 3000 using the collected plasma as a gradient. The washed PBPC was thereafter transferred into a PL-732 blood container (Baxter) and when required stored in sterile conditions overnight at room temperature and pooled together with the platelet washed PBPC collected the day after.

The CD34+ cell enrichment was generally done employing the ISOLEX 300I device 1.12 or 2.0 (Baxter, Irvine, CA, USA). Only in one patient was CD34+ cell selection performed by an ISOLEX 300SA using Chymo-Cell as a releasing agent. PBPC and purified CD34+ cells were prepared and control-rate frozen following a standardised procedure.[29] For the patients given purified CD34+ cells, unmanipulated PBPC containing $\geqslant 2 \times 10^6$ CD34+ cells/kg were frozen and stored, as a security measure.

Enumeration of CD34+ cells and colony-forming unit analysis

Total nucleated cells (TNC) in the PBPC products were counted in a Cell dyn 3500 cell counter (Abbott Laboratories, Irvine, CA, USA). Enumeration of CD34+ cells in blood and in the apheresis product was determined by use of a FACSort flow cytometer (Becton Dickinson, San Jose, CA, USA) using the Nordic standardisation method.[30] The colony-forming unit-culture (CFU-c) assay was performed employing a standardised method with media containing appropriate amounts of growth factors (Methocult H4433; Stem Cell Technologies, Vancouver, Canada).

High-dose therapy, stem cell transplantation and treatment post transplant

As conditioning regimens, 36 patients received BEAM (carmustine 300 mg/m^2, etoposide 300 mg/m$^2 \times 4$, cytarabine 400 mg/m$^2 \times 4$ and melphalan 140 mg/m^2) from day −7 to day −3, three patients BEAC (carmustine, etoposide, cytarabine, cyclophosphamide) and one patient cyclophosphamide and total body irradiation (TBI) in fractionated doses (1.3 Gy twice daily during 5 days) for a total of 13 Gy. The stem cell products (enriched CD34+ cells or unmanipulated PBPC) were rapidly thawed in a 37°C water-bath and infused on day 0. According to national guidelines, G-CSF was not used routinely after HDT. Twenty of the 25 patients, who entered transplantation with clinical or radiological evidence of disease, received additional therapy after the HDT; 18 patients received involved-field radiotherapy and two patients were splenectomised.

Supportive care

As *Pneumocystis carinii* prophylaxis trimethoprim/sulfamethoxazole was started during the HDT regimen and was continued for 3 months after transplantation. Nystatin mixture was given prophylactically until the neutropenic period was over. Acyclovir was started at day −3 and continued for 3 months to prevent herpes virus infections. From January 1999, antibiotic and antiviral treatment was given for 1 month only. Broad-spectrum intravenous antibiotics and antifungal therapy were administered according to a standardised protocol for febrile neutropenic episodes. Twenty-six (65%) of the patients were cytomegalovirus (CMV) seropositive. CMV-negative blood products were given to CMV-negative patients. Blood products were filtered and irradiated (30–35 Gy) before infusion to avoid problems with alloreactivity. All patients were examined regularly before HDT, weekly after reinfusion of stem cells until discharge and at 3, 6 and 12 months after HDT for CMV infections with serum analysis of CMV very early antigen or, from 1997, detection of CMVpp65 antigen in blood leukocytes.

Flow cytometric analysis of immunoreconstitution

Mononuclear cells from peripheral blood of HD patients were prepared by centrifugation on a Ficoll–Isopaque gradient. Two-colour immunofluorescense was performed and the cells were analysed on a FACscan flow cytometer. Data acquisition and analysis were performed using the Cell Quest software (Becton Dickinson). For enumeration of lymphocyte subsets, only cells within the lymphocyte gate based on the light scatter properties were included in the analysis. The percentage of B cells (CD19 + CD20)/2, CD56+ cells (includes NK cells and T cells positive for CD56), T cells (CD3) and T cell subsets (CD4, CD8 and CD4/CD8 ratio) were analysed. Irrelevant isotype-matched fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-labelled monoclonal antibodies were used as negative controls. The monoclonal antibodies CD19 PE, CD20 PE, CD3 FITC, CD4 FITC and CD8 PE were obtained from DAKO (Glostrup, Denmark) and the CD56 PE from Becton Dickinson. For comparison, peripheral blood from 17 healthy donors was also analysed for lymphocyte subsets and used as controls.

Immunoglobulin levels

Quantification of IgA, IgM and IgG including IgG subclasses (IgG1, IgG2, IgG3 and IgG4) was performed by nephelometry, using a BN II analyzer and reagents from Dade Behring (Liederbach, Germany) at The National Hospital, Oslo, Norway. Analyses were performed at 1–2 months before HDT and 3 and 12 months after HDT.

Infectious episodes after HDT

Since some previous reports have indicated that the use of CD34+ cells gave a higher number of severe infections after HDT, the patients clinical records of follow-up visits at the hospital at 3, 6, 9 and 12 months after HDT were reviewed.

In addition, all patients who were alive (PBPC group (*n* = 15), CD34 group (*n* = 18)) as of 1 September 2000 were sent a questionnaire regarding infectious episodes during the first 3 months after the hospitalisation period as well thereafter.

Evaluation and response criteria

Before HDT, all patients were restaged and underwent physical examination, chest radiography, abdominal and pelvic CT scanning (and chest CT scanning if indicated), ultra sound of upper abdomen and liver /spleen and bone marrow biopsy. CR was defined as the complete disappearance of all measurable radiographic or clinical evidence of disease. PR was defined as a reduction in tumour mass by more than 50%, measured as the sum of the product of the two largest perpendicular diameters of the tumours. Progression was defined as at least 25% increase in previously noted tumour mass or the appearance of any new additional tumour. Response to HDT was assessed 3 months after the date of stem cell reinfusion. Routine follow-up with restaging was carried out every 3 months the first year, every 4 months the next year and every 6 months up to 5 years and then yearly after HDT or whenever clinically indicated.

Statistical analysis

Statistical analysis was performed using the SPSS 9.0 for Windows (SPSS, Chicago, IL, USA). The analysis was performed on all data obtained as of 9 July 2001. Event-free survival (EFS) was calculated from the date of HDT to the date of progression, treatment-related death, or date of last follow-up. Overall survival (OS) was measured from the date of HDT to the date of death from any cause or date of last follow-up. EFS and OS were estimated using the product-limit method of Kaplan–Meier. The two groups, CD34$^+$ selected and unselected PBPC, were compared with the Mann–Whitney test. The log-rank test was used to compare the survival curves of the two groups of patients. Correlations were done with multiple linear regression analysis. *P* values <0.05 were considered as statistically significant.

Results

Engraftment

Following HDT, the patients receiving PBPC were reconstituted with a median number of 4.5×10^6 (range 2.3–17.6) CD34$^+$ cells/kg while the CD34$^+$ selected group received a median number of 5.8×10^6 (range 2.7–20) CD34$^+$ cells/kg (Table 2). At the discretion of the attending physician, five (26%) of the patients in the PBPC group and three (14%) in the selected CD34$^+$ cell group were given G-CSF after HDT starting at median day 7 (range 1–10) and median day 15 (range 13–20), respectively. All patients engrafted, and none of the patients in the CD34$^+$ group were given additional back-up of unmanipulated PBPC. No differences were seen between the two HD groups regarding time to engraftment, days with fever ⩾38.5°C, number of post-transplant blood transfusions or days in hospital (Table 2).

Table 2 Characterisation of the autografts and engraftment data

Number of patients	*PBPC* *n* = 19	*CD34$^+$* *n* = 21
Before manipulation		
TNC × 10^9	24.5 (7.7–80)	20.3 (13.27–67.0)
CD34 × 10^8	4.5 (2.3–17.6)	9.2 (3.8–13.9)
After manipulation		
CD34 × 10^8 in selected fraction		4.05 (1.5–10.9)
Purity %		99.2 (97.8–99.7)
Yield %		49.6 (30.0–78.4)
Reinfusion		
TNC × 10^9	24.5 (7.7–80.0)	0.375 (0.15–11.0)
CD34$^+$ cells × 10^6/kg	4.5 (2.3–17.6)	5.8 (2.7–20.0)
CFU-c × 10^4/kg	105.5 (46.0–1360.0)	127.5 (64.0–224.0)
Engraftment		
Days to ANC ⩾0.5 × 10^9/l	13 (9–29)	13 (10–20)
Days to platelet count ⩾20 × 10^9/l	12 (9–18)	10 (7–20)
Days to platelet count ⩾50 × 10^9/l	15 (9–50)	14 (10–28)
No. of patients using G-CSF after HDT	5	3
No. of patients with fever ⩾38.5°C	18	21
Days with fever ⩾38.5°C	4 (0–14)	4 (1–9)
Days on i.v. antibiotics	10 (0–18)	11 (4–26)
Days in isolation	12 (10–14)	12 (10–16)
Days in hospital	17 (14–41)	15 (13–30)
No. of platelet units transfused	10 (2–64)	8 (2–40)
No. of RBC units transfused	6 (2–16)	6 (0–12)

TNC = total nucleated cells; CFU-c = colony-forming unit-culture; ANC = absolute neutrophil count; RBC = red blood cell; values are given as median and range.

Immunological reconstitution

At 3 and 12 months after HDT, the total number of lymphocytes in the CD34$^+$ group was a median of 4.6×10^9/l (range 0.3–9.5) and 4.9×10^9/l (range 1.0–7.7), respectively. In the PBPC group, the corresponding median numbers were 4.7×10^9/l (range 0.6–8.4) and 4.0×10^9/l (range 0.6–13) lymphocytes, respectively. In all but three patients, this was above the normal lower range ($0.9–4.0 \times 10^9$/l) (Figure 1). No significant differences in total lymphocyte counts were seen between the two HD groups at 3 months (*P* = 0.8) and 12 months (*P* = 0.2).

Moreover, we found no important differences between the HD groups regarding the distribution of lymphoid subsets (Figure 2a–e).

The percentage of B cells was significantly higher in the CD34$^+$ group than in the control group 3 and 12 months after HDT (*P* < 0.05). No such difference was seen between the PBPC group and control group at 3 months (*P* = 0.63), while at 12 months the value was also significantly higher (*P* < 0.05) (Figure 2a).

The CD56$^+$ cells were reconstituted at a normal level in the two HD groups, except for a lower (*P* = 0.04) CD56$^+$ percentage in the PBPC group at 12 months (Figure 2b).
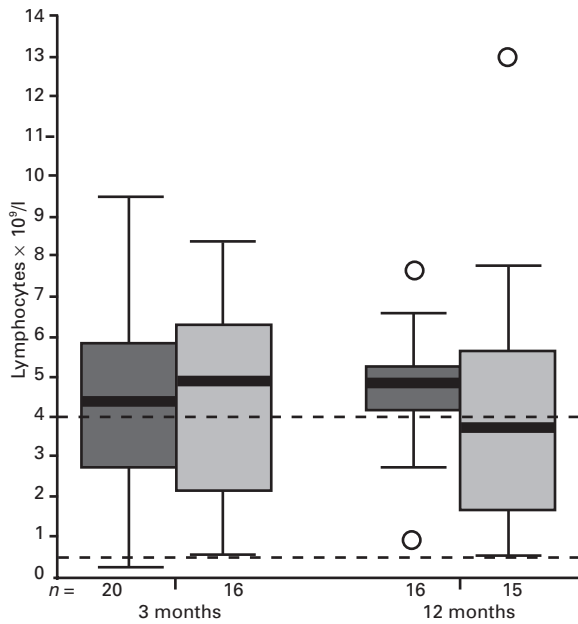
Figure 1 Total number of lymphocytes 3 and 12 months after HDT, CD34+ (■) or PBPC (▨). Normal reference laboratory values are between the horizontal dotted lines. The boxes include the median value, 25% and 75% fractils, while the error bars denote the minimum and maximum values, except the outliers that are marked.

In both study groups, the recovery of total T cells (CD3+ cells) was delayed when analysed at 3 and 12 months (Figure 2c). When the CD3+ cell values in the HD patients were compared to the controls, the CD3+ cell values were significantly lower in the CD34+ group at 3 months ($P < 0.05$), 12 months ($P < 0.05$), and in the PBPC group at 12 months ($P < 0.05$). The lower value of CD3+ cells at 3 months for the PBPC group was of borderline significance compared to the controls ($P = 0.05$). The lower CD3+ values were exclusively due to low CD4+ T cell numbers in both study groups compared to controls. No significant differences were found between the groups at 3 months ($P = 0.29$) and 12 months ($P = 0.27$) (Figure 2d). The percentage of CD8+ T cells was increased at 3 months in both groups, and slightly declined at 12 months (Figure 2e). Due to the low CD4+ cell number, an inversion of the CD4/CD8 ratio was seen at 3 months in the PBPC as well as the CD34+ group, giving median values of 0.4 (range 0.2–0.7) and 0.5 (range 0.1–1.7), respectively. This was significantly lower than the control group: 1.4 (range 0.5–5.0), ($P < 0.0001$ and $P < 0.0001$). Although the CD4/CD8 ratio slightly improved at 12 months in both groups, a median of 0.6 (0.3–1.0) for the PBPC group *vs* a median of 0.7 (0.2–1.2) for the CD34+ group, it remained significantly lower than that of the controls ($P < 0.0001$ and $P < 0.0001$), respectively. When the CD4/CD8 ratio at 3 and 12 months was compared between the HD groups no significant differences were seen.

No significant correlation was seen between CD4+ T cell values and age or number of CD34+ cells infused for all of the patients, nor for the HD groups when analysed separately.

One to 2 months before and 3 and 12 months after HDT, serum immunoglobulin levels of IgA, IgM, IgG (Figure 3) and IgG subclasses (IgG1, IgG2, IgG3 and IgG4) (data not shown) were, for the majority of patients, within the normal range in both HD groups. One patient in the CD34+ group with a history of colitis ulcerosa, had a low level of IgA both before, 3 and 12 months after HDT.

Infectious episodes after HDT

All patients in both groups except one patient in the PBPC group had episodes of fever within the first 30 days after HDT and were given i.v. antibiotics. Infections or bacteremias were documented in nine patients (38%) treated with purified CD34+ cells, of whom two patients had more than one infectious episode: *Streptococcus viridans* ($n = 5$), *Staphylococcus epidermidis* ($n = 2$), *Enterobacter* ($n = 2$), *Klebsiella pneumonia* ($n = 1$), *E. coli* ($n = 1$). Six patients (32%) who were given unmanipulated PBPC had documented infections, of whom three had more than one infectious episode: *Streptococcus viridans* ($n = 1$), *Streptococcus pneumonia* ($n = 1$), *Staphylococcus epidermidis* ($n = 3$), *Klebsiella pneumonia* ($n = 1$), *E. coli* ($n = 1$), *Pneumocystis carinii* ($n = 1$). One patient in the PBPC group had appendicitis, which was treated conservatively. No patient had detection of CMV pp65 antigen in blood leukocytes and no clinically apparent CMV infections were observed. None of the patients had documented fungemia. Within the first 30 days after HDT, we found no significant difference in microbiologically documented infections between the two HD groups ($P > 0.1$).

The number of infectious episodes after hospitalisation were based on the evaluation of clinical records during regular follow-up visits and on the patients' reply of the questionnaire (93% replied in the PBPC group and 94% replied in the CD34+ group). As can be observed in Table 3, there was no difference in the given number of infections between the two groups, either during the first 3 months or after 3 months from discharge from hospital.

Survival

A total of 19 patients (90%) in the CD34+ group and 17 patients (89%) in the PBPC group achieved or maintained a CR after HDT. The estimated OS at 4 years was 86% for the CD34+ group and 74% for the PBPC group, with a median follow-up of 37 months (range 1–61) and 46 months (range 4–82), respectively ($P = 0.9$) (Figure 4). The estimated EFS at 4 years showed no difference between the CD34+ group and the PBPC group: 75% *vs* 63%, respectively ($P = 0.4$) (Figure 5). Four patients (19%) in the CD34+ group and six (32%) in the PBPC group had a relapse or progression after HDT ($P = 0.4$).

Two patients (5%) died as a result of complications related to the HDT. One patient reconstituted with purified CD34+ cells died 1 month after transplantation from respiratory failure after developing acute respiratory distress syndrome (ARDS). The patient who was given unmanipulated PBPC, developed heart and kidney failure and died 13 months after HDT without evidence of relapse of HD. Overall, 10 patients relapsed at a median of 10 months (range 3–28) after HDT, generally at sites of previous dis-
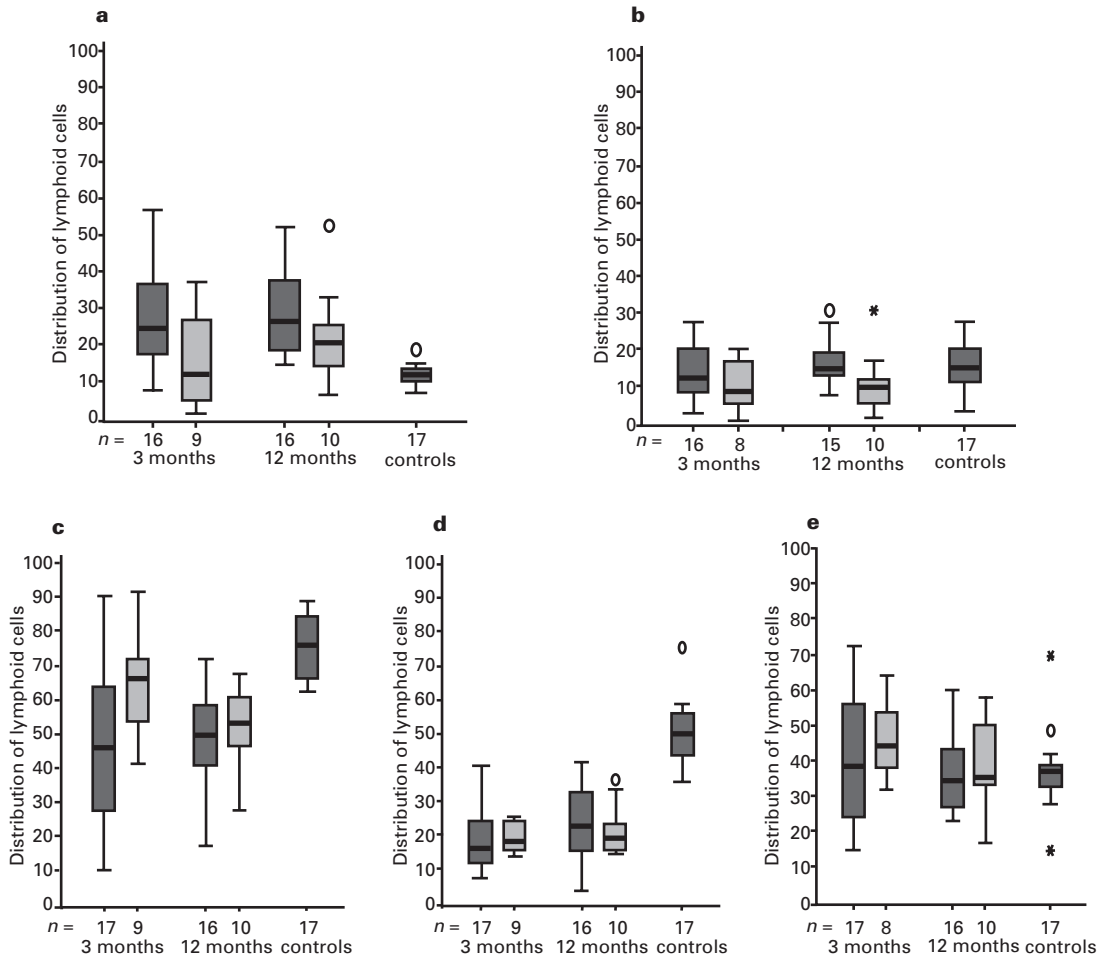
Figure 2 Distribution of lymphoid cells in percent of cells in lymphocyte gate after HDT; CD34+ (■), PBPC (▨) or controls (▨). (**a**) B cells (CD19 + CD20)/2, (**b**) CD56+ cells, (**c**) T cells (CD3), (**d**) T cells (CD4), (**e**) T cells (CD8). The boxes include the median value, 25% and 75% fractils, while the error bars denote the minimum and maximum values, except the outliers that are marked.
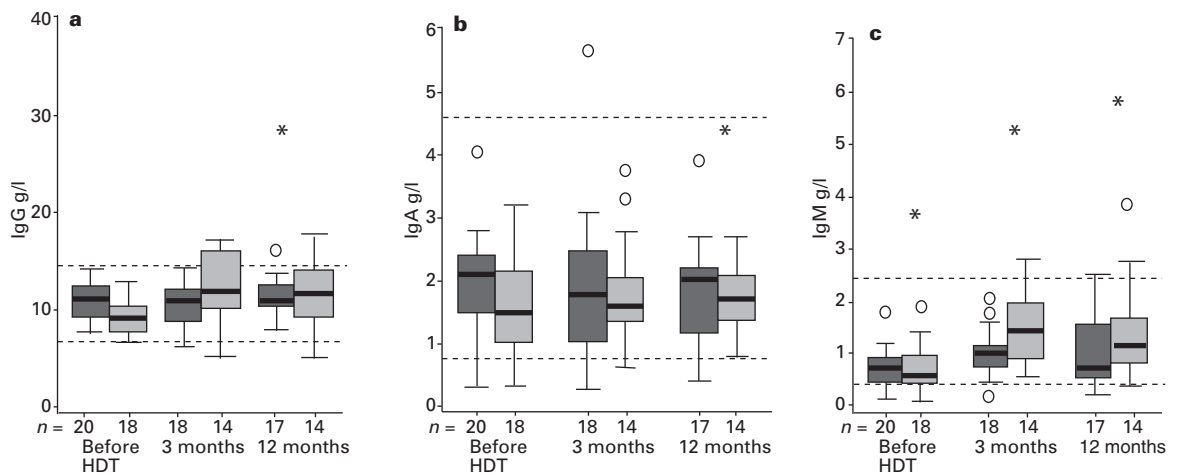

Figure 3 Immunoglobulin levels (g/l) before HDT, 3 and 12 months after; CD34+ (■) or PBPC (▨). Normal reference laboratory values are between the horizontal dotted lines. (**a**) IgG, (**b**) IgA, (**c**) IgM. The boxes include the median value, 25% and 75% fractils, while the error bars denote the minimum and maximum values, except the outliers that are marked.

Table 3 Infectious episodes after discharge from hospital after HDT based on clinical records of follow-up visits and patients answers of a questionnaire

	PBPC		CD34+	
	No.	(%)	No.	(%)
No. of patients alive who were sent the questionnaire	15		18	
No. of patients answering the questionnaire	14	(93)	17	(94)
Infections day +30 to day +90 after HDT				
Severe, requiring hospitalisation				
FUO	1	(7)	0	
Bacterial pneumonia	0		1	(6)
Mild				
Common cold/sinusitis/bronchitis/UTI	5	(33)	3	(17)
Herpes simplex	1	(7)	0	
Infections >day +90 after HDT				
Severe, requiring hospitalisation				
Pneumonia (*Pneumocystis carinii*)	1	(7)	0	
Fungal pneumonia (*Aspergillus*)	1	(7)	0	
Mild				
Common cold/sinusitis/bronchitis/pneumonia/UTI	6	(40)	11	(61)
Herpes zoster	5	(33)	5	(28)
Cutaneous/mucous fungal infections	4	(27)	4	(22)
Patients' sensation of more infections after HDT compared to before HDT	7	(47)	6	(33)

FUO = fever of unknown origin. UTI = urinary tract infection.
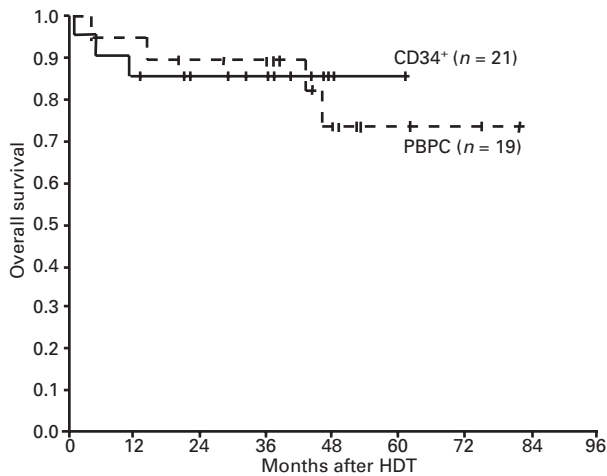
Figure 4 Overall survival after HDT of the 40 HD patients given either selected CD34+ cells or unmanipulated PBPC.

ease. Two of the 10 patients who relapsed, achieved a new CR with chemotherapy and/or irradiation. Five patients are still alive at a median of 27 months (range 1–62) after relapse. Eight of the 25 patients (32%) in PR at the time HDT, relapsed or progressed after treatment. In contrast, only two of the 15 patients (13%) in CR at the time of HDT relapsed ($P = 0.2$).

Discussion

The use of CD34+ cell enriched grafts might be of significance in order to prevent relapse in HD patients treated with HDT and stem cell support.[14,15] However, of major
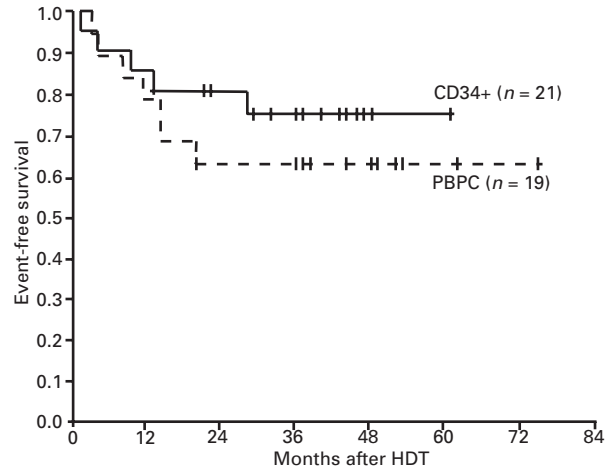
Figure 5 Event-free survival after HDT of the 40 HD patients given either selected CD34+ cells or unmanipulated PBPC.

concern are the existing data in different malignancies indicating that the haematological and immune reconstitution are impaired to a greater extent after the use of selected CD34+ cells as compared to the use of unmanipulated PBPC.[18–21] In this study, we report the clinical findings in a uniform group of patients with HD who received either purified CD34+ cells or unmanipulated PBPC. The haematological and immune reconstitution in both groups was rapid with normal levels of neutrophils, platelets, lymphocytes, immunoglobulins, CD56+ cells, and an increased level of B cells and CD8+ T cells at 3 months. The percentage of CD3+ T cells was low, primarily due to the delayed reconstitution of CD4 T cells, which led to an inverse CD4/CD8 ratio which also persisted 12 months post transplantation. Importantly, we did not observe significant differences in the reconstitution of any of these cell types between patients receiving either purified CD34+ cells or PBPC as stem cell support, except for somewhat lower levels of CD56+ cells in the PBPC group at 12 months.

With the exception of some previous reports which showed a slower platelet recovery after the use of CD34+ selected stem cells,[5,21] most studies experienced no differences in haematological recovery between the use of selected CD34+ cells or unmanipulated PBPC.[31–33] We observed a fast haematological engraftment in all patients after reinfusion with a median number of 5.8×10^6 CD34+ cells/kg in the CD34+ group and 4.5×10^6 in the PBPC group. In addition, the engraftment data indicate that G-CSF is not necessary after HDT when a satisfactory number of CD34+ cells are reinfused.

Importantly, the immune reconstitution was similar in both HD groups. At 3 and 12 months after HDT, the absolute lymphocyte count, the B cell (CD19, CD20), T cell (CD3, CD4, CD8) and CD56+ cell levels at 3 months were not different in the two groups (Figures 1 and 2). In contrast to some previous reports,[34,35] we found normal immunoglobulin levels at both 3 and 12 months in both groups. The median values of the absolute lymphocyte number in both groups at 3 and 12 months were above the upper normal range. Thus, the high percentage of B cells observed at both 3 and 12 months most likely reflects an increased

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number of B cells present. Similar results have also been observed by Mackall *et al*[35] in children and young adults giving a median of 7.2×10^6 CD34+ cell/kg.

Some studies are in accordance with our results,[3,22–24] while others indicate that the use of enriched CD34+ cells gives delayed lymphoid reconstitution.[18–21] One possible explanation for the different results obtained, is that as for the granulocyte and platelet recovery, a minimum number of CD34+ cells/kg has to be infused to achieve an optimal immune reconstitution.[23,24,35] In particular, Bomberger *et al*[18] found that when a mean number of 1.8×10^6 selected CD34+ cells/kg were infused, both recovery of T and B cells were severely affected during the first year after transplantation. T cell receptor (TCR) studies also showed decreased diversity of the peripheral T cell repertoire in the CD34+ group compared to unmanipulated PBPC.

Mackall *et al*[35] observed a relationship between age and CD4+ reconstitution, but interestingly no correlation between CD4+ cell reconstitution and numbers of CD34+ cells infused was found. Although, our patients who received selected CD34+ cells were significantly younger than the patients in the PBPC group, we were unable to detect a linear relationship between CD4+ cell reconstitution and age or number of CD34+ cells reinfused. The patients in our study and that of Mackall *et al*[35] were given a relatively high median number of CD34+ cells. Therefore, the lack of linearity between CD34+ cells infused and lymphoid reconstitution, might be due to the numbers of infused CD34+ cells are above the threshold required to see differences in the lymphoid reconstitution.

Three main epitope classes have been characterised for the CD34 molecule.[36] We have used the Isolex 300I System for CD34+ selection with a class II type antibody, while most of the other groups have applied the Ceprate System[37] in which they use a class I type antibody. Thus, the enrichment procedure used could potentially select for different subsets of CD34+ cells, which in turn might influence the capability of immune reconstitution.

Furthermore, the immune reconstitution might differ according to type of malignancy. In our study, a uniform type of patients with HD has been studied while previous reports have studied different types of malignancies. Age, as discussed earlier, could also be important. The Hodgkin patients in our study were considerable younger (median 25 years), than the patients in the other studies who had a median age above 40 years.

It was also important to determine clinical parameters related to immune reconstitution in these patients. It is not settled whether the use of CD34+ cell selection causes a higher incidence of post-transplant infections.[3,19,21,23,24,38] We found no difference between the HD groups in the number of infections, days with fever, days on i.v. antibiotics during the first 30 days after HDT. Furthermore, the number of infections after discharge from hospital was comparable between the groups. A severe fungal infection was observed in only one patient (PBPC group). Some data indicate a relationship between CMV infection and the use of purified CD34+ cells infused.[20,39] We did not observe any clinical CMV infections either in the CD34+ group or in the PBPC group in our study.

The aim of this study was to examine the engraftment, immune reconstitution and infections in HD patients receiving purified CD34+ cells as stem cell graft. The method is, according to our findings, safe and comparable to unmanipulated PBPC. Any conclusion on improved survival using CD34+ cells are premature as the observation time is still short and differs between the groups, and, unlike in non-Hodgkin's lymphomas, even late relapses may occur after HDT. A potential prognostic difference between the two groups can only be confirmed by a larger prospective randomised trial.

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