Gene Ther Mol Biol Vol 11, 21-26, 2007

Autologous stem cell transplantation for primary refractory or relapsing Hodgkin's disease: comparison between CD34$^+$ immunoselected and unselected stem cells graft

Research Article

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Key words: autologous stem cell transplantation, CD34$^+$, cell immnuoselection, Hodgkin's disease

Abbreviations: Autologous stem cells transplantation, (ASCT); Bloodstream infection, (BSI); Centre for Disease Control, (CDC); Complete response, (CR); disease free survival, (DFS); freedom from progression, (FFP); Hodgkin's disease, (HD) either; leukapheretic procedure, (LKP); non-prophylactic antibiotics, (NPA); Overall survival, (OS); Partial response, (PR); peripheral blood stem cells, (PBSC); qualitative chain reaction, (PCR)

Received: 28 November 2006; Revised: 01 February 2007
Accepted: 05 February 2007; electronically published: March 2007

Summary

Autologous stem cells transplantation (ASCT) is widely accepted in the treatment of high risk Hodgkin's disease (HD) either resistant or relapsing after first-line chemotherapy. Since the presence of Hodgkin/Reed- Sternberg cells in peripheral blood stem cells (PBSC) collection was demonstrated, and CD34$^+$ antigen is not expressed on HD cells, positive selection of CD34$^+$ cells has been demonstrated an efficient purging method to reduce the number of tumour cells in the graft. We conducted a non randomized pilot study using CD34$^+$ selected ASCT in refractory/relapsing HD patients and compared the clinical outcome to HD patients receiving unselected PBSC. Eleven patients received CD34$^+$ selected ASCT (group A) and 11 patients received unmanipulated ASCT (group B). Patients were matched for age, sex, diagnosis, IPI and response to first-line therapy. Group A received a median number of 4.74 x 10^6/kg CD34$^+$ selected cell while patients of B group of 8.45 x 10^6/kg unselected PBSC (p=0.23). No difference was observed between the two groups in any of the endpoints analyzed (haematological engraftment, infections, morbidity and mortality, progression, survival). These results show no advantages for patients receiving CD34$^+$ selected ASCT compared to unselected PBPC transplant at least for refractory/relapsing HD.

I. Introduction

Autologous stem cells transplantation has been included in the algorithm of treatment of high risk Hodgkin's disease. In the last years, peripheral blood stem cells (PBSC) have replaced bone marrow as stem cell source because of a faster haematological recovery after reinfusion but some authors suggested a less favourable outcome in patients receiving PBSC compared to bone marrow (Maiolino et al, 1997). An explanation for these results might originate in the presence of neoplastic contamination of peripheral stem cells collection (Wolf et al, 996) although the correlation between the presence of tumour cells in the grafts and the incidence of relapse after high-dose therapy is currently unsettled.

Classic HD cells express CD30 and CD15 antigens on their surface and lack the expression of CD34 antigen. The positive selection of CD34$^+$ cells from PBSC has been demonstrated an efficient purging method to reduce the number of atypical CD30$^+$ cells (Blystad et al, 2001a,b) in peripheral blood stem cells collection from patients with HD. However the advantage in terms of clinical outcome in patients receiving CD34$^+$ selected PBSC compared to those receiving unselected PBSC has not been established. In order to elucidate the role of purging in HD we report the results of haematological reconstitution and clinical outcome of patients receiving CD34$^+$ selected PBSC

transplantation compared to a well-matched group of high risk patients receiving unmanipulated PBSC.

II. Materials and Methods

The majority of HD were reconstituted with unmanipulated PBSC after high dose chemotherapy, but from march 1996 a pilot study of $CD34^+$ selection and ASCT was started and positive selection of the PBSC was performed in all patients with poor prognostic features such as refractory or relapsing Hodgkin's disease with or without bone marrow involvement. The protocol was approved by the local ethical commitee and patients or their guardians gave their informed written consent. Eleven patients (group A) were enrolled onto the study which was opened at the beginning of 1996 and closed at the end of 1999. For the purpose of the analysis patients were then matched for demographic and clinical parameters to a group of patients who received unmanipulated PBSC (group B) from March 1992 to October 2001 with Hodgkin's disease autografted at our Institution and extracted from our database.

Characteristics of patients are showed in **Table 1**. Median age was 23 years (range 17-42) in group A and 30 years (range 15-35) in group B; sex distribution was: 4 males and 7 females in group A and 6 males and 5 females in group B. The median IPI score at diagnosis calculated according to Bierman et al (Bierman et al, 2002) was 2 in both groups with a range of 1-3 in group A and 1-4 in group B respectively. Disease stage at diagnosis was III-IV in the majority of patients: 7 of 11 patients (64%) had IV stage in the group A and 5 of 11 (45%) in group B and the vast majority (9 out of 11 patients: 81%) had B symptoms in both groups. Nodular sclerosis was the histological subtype in 8 of 11 patients (73%) in both groups. Bone marrow involvement was present in 5 (45%) patients in group A and in 3 patients (27%) in group B.

All patients at diagnosis received conventional first-line therapy as reported in table 1. Complementary radiotherapy was administered in 4 patients (36%) in group A and 1 patients (9%) in group B either after first line chemotherapy or for relapse. Group A included 2 patients (18%) in relapse at 6,5 months (range 3-10), 2 patients (18%) achieving partial remission after first line chemotherapy and 7 patients (63%) with disease progression, Group B included 3 patients (27%) in relapse at 16 (range 3-60) months, 2 patients (18%) achieving partial remission after first line chemotherapy and 6 patients (54%) with disease progression.

A. Salvage chemotherapy

All patients were treated with salvage chemotherapy: mitoxantrone 10 mg/m^2 day 1, carboplatinum 100 mg/m^2 day 1-4, cytosine arabinoside 2 g/m^2 day 5, methylprednisolone 500 mg/m^2 day 1-5 (MiCMA) (La Barbera et al, 2000) followed by G-CSF and peripheral blood stem cell collection. After evaluation of response to chemotherapy, autologous stem cell transplantation was carried out in all patients irrespective of their response.

B. Response to salvage chemotherapy and disease status at transplantation

Before transplantation all patients underwent accurate restaging by total body CT scanning and bone marrow biopsy. Complete response (CR) was defined as complete disappearance by physical exam and radiographic studies of all measurable or evaluable disease with no indication of recurrence. Partial response (PR) was defined as ≥ 50%, but < 100% improvement in all measurable or evaluable disease, with no indication of tumour regrowth before high dose chemotherapy consolidation. After a median of 2 cycles (range 1-4) of MiCMA, response and thus, disease status at transplantation, was as follows: in group A, CR in 2 (18%)patients, PR in 5 (45%) patients and 4 (36%) patients showed disease progression; in group B, 2 (18%) patients obtained CR, 6 (55%) patients achieved PR and 3 (27%) patients had disease progression.

C. $CD34^+$ cell harvesting and purging

PBSC were mobilized and collected after MiCMA plus G-CSF 5mg/kg/day subcutaneously (sc) from day 8 until completion of leukapheretic procedure (LKP). Leukapheres started when the peripheral white blood count was greater than 1.0 x 10^9/l and CD34+ cells in peripheral blood were >20/ml. LKP were continued daily until a minimum harvest of 2x10^6 $CD34^+$/kg was obtained. In patients submitted to immunoselected $CD34^+$ stem cell transplantation the selection was carried out using the Ceprate SC system (Cellpro, Bothell, WA, USA) or the CliniMACS device (Miltenyi Biotech GmbH, Bergish-Gladbach, Germany). All patients had a back up of unfractionated PBSC stored in liquid nitrogen.

D. Stem cell transplantation

As conditioning regimen all patients in both groups received BEAM (carmustine 300mg/m2on day –7, etoposide 200mg/m2 on day-6,-5,-4,-3, cytosine arabinoside 200mg/m2 on day-6,-5,-4,-3 and melphalan 140mg/m2 on day –2). In nine out of 11 patients receiving $CD34^+$ selected PBSC, G-CSF was administered at a standard dose of 263 mg daily or on alternate day until neutrophil count was >500/ml for two consecutive days. All patients received standard antimicrobial, antiviral treatment and Pneumocystiis Carinii prophylaxis. Broad spectrum intravenous antibiotic therapy was started for fever of unknown origin in neutropenic patients.

Empirical antifungal therapy was started for fever not responsive to antibiotics therapy. All patients were CMV seropositive and were regularly examined for CMV infection after transplantation with antigenemia or qualitative chain reaction (PCR) for CMV DNA.

Bloodstream infection (BSI) and catheter related infection were defined according to Centre for Disease Control (CDC) (CDC-MMWR, 2002).

Viral infections were defined as the evidence of positive colture from urine, stools or throat in association with symptoms. CMV infection was defined as either the evidence of any level of pp 65 antigenemia and or a positive culture.

E. Endpoints

During this study we evaluated the following clinical outcomes: time to neutrophil recovery (0.5x10^9/l and 1.0x10^9/l), time to platelets recovery >20x10^9/l and >50x10^9/l, reticulocyte recovery (>1%), time to untransfused Hgb >10 g/dl, number of pRBCu and SDu infused, length of hospitalization, duration of non-prophylactic antibiotics (NPA), number of days with BT>38°C, incidence of sepsis and viral infections, duration of G-CSF administration (in days), overall survival, freedom from progression and disease free survival for patients achieving complete response.

F. Statistical analysis

Overall survival (OS) analysis, disease free survival (DFS) and freedom from progression (FFP) analysis were assessed from the first day of transplantation (version 2.01; GraphPad Software Inc., San Diego, CA).

In OS analysis, an event was defined as death from any cause; the DFS was calculated for all patients who had reached CR, FFP was calculated from the date of response until relapse or progression whichever came first. Survival curves were generated using Kaplan-Meier method for survival analysis and log-rank test was performed for survival curve comparison. Chi2

test was used to analyse the categorical factors. Statistical significance was defined as $p<0.05$.

Table 1. Characteristics of patients

Characteristics	**PBSC** **No**	**CD 34$^+$** **No**
No. of patients(%)	11 (100)	11 (100)
Sex (%): **Male** Female	 4 (36) 7 (64)	 6 (55) 5 (45)
Age, median (range) in year	23 (17-42)	30 (15-35)
Histology (%): Nodular sclerosis Nodular sclerosis1 Nodular sclerosis2 Mixed cellularity Lymphocyte depletion HD- not otherwise specified	 5 (45.5) 1 (9) 2 (18.25) 0 (0) 2 (18.25) 1 (9)	 6 (55) 0 (0) 2 (18) 3 (27) 0 (0) 0 (0)
Staging (%): I-II III-IV unknown	 3 (27) 7 (64) 1 (9)	 1 (9) 10 (91)
B symptoms (%)	9 (81)	9 (81)
Bone marrow involvement (%) unknown	5 (45) 1 (9)	3 (27)
Bulky disease (%) unknown	7 (64)	2 (18) 2 (18)
IPI (%): I II III IV	 5 (45.5) 2 (18.25) 4 (36.25) 0 (0)	 5 (45.5) 2 (18.25) 3 (27.25) 1 (9)
First-line chemotherapy (%): ABVD ABVD/MOPP VEPEB BEACOPP STANFORD V MOPP	 5 (45.5) 3 (27.25) 2 (18.25) 1 (9)	 5 (45.5) 1 (9) 0 (0) 0 (0) 2 (18.25) 3 (27.25)
Radiotherapy (%):	4 (36)	1(9)
Response to first-line therapy (%): PD CR PR	 6 (55) 3 (27) 2 (18)	 6 (55) 3 (27) 2 (18)
Status at transplantation (%): **PD** PR CR	 4 (36.25) 5(45.5) 2 (18.25)	 3 (27) 6 (55) 2 (18)
CD34$^+$ cells reinfused x 10 ^{6} (range) :	4.74 (1.39-13.8)	8.45 (1.99-19.7)
Engraftment (range) : Days to ANC >0.5x10^9/l Days to ANC >1.0x10^9/l Days to haemoglobin level >10g/dl Days to reticulocyte>1% Days to platelet count >20x10^9/l Days to platelet count >50x10^9/l Days with fever>38°C Days in hospital No.of platelet units transfused No.of RBC units transfused	 12(9-20) 13(9-40) 45(14-120) 15(9-34) 13(10-33) 16(13-120) 4(2-20) 27(21-46) 2(0-4) 2(0-6)	 13(10-28) 17(10-60) 23(5-90) 14(11-90) 11(7-19) 18(10-60) 4(0-15) 27(21-40) 2(0-4) 1(0-6)

CR= complete remission, PR= partial remission, PD= progressive disease, ANC= absolute neutrophil count, RBC= red blood cell, IPI= international prognostic index.

III. Results

A. Haemopoietic recovery

After conditioning chemotherapy the patients in group A received a median number of 4,74 x 10^6 (range 1.39-13.8) $CD34^+$ cells/kg while the patients in group B received a median number of 8,45 x 10^6 (range 2.28-13.7) $CD34^+$ cells/kg (p= ns).

Nine out of 11 patients receiving $CD34^+$ selected cells received G-CSF after reinfusion for a median number 11 doses (range 4-15). No patients received G-CSF in the B group. All patients engrafted, no patient needed additional back-up of unmanipulated PBSC.

The median time to recover a neutrophil count >0.5 x 10^9 and > 1 x 10^9 was 12 days (range 9-20) and 13 days (range 9-40) respectively in A group and 13 days (range 10-28) and 17 days (range 10-60) respectively in B group (p=ns).

Median time to achieved a self-sustained platelet count> 20 x 10^9/l and > 50x 10^9/l in the patients of A group was 13 (range 10-20) and 16 days (range 13-120) respectively (p=0.04) and 11 (range 8-19) and 18 days (range 10-60) respectively in patients of B group.

Median time to achieve a reticulocyte count > 1% in the patients of A group was 15 days (range 9-34) and 14 days (range 11-90) in patients of B group (p=ns). Haemoglobin level > 10 g/dl was reached after 45 days (range 14-120) in the patients of A group and after 23 days (range 5-92) in the patients of B group (p=ns).

Median length of hospitalization was 27 days in both groups (p=ns).

No statistically significant difference was observed in terms of transfusion requirements: patients of A group received a median number of 2 SDu of platelets (range 0-4) and 2 pRBCu (range 0-6); patients of B group received a median number of 2 SDu of platelets (range 0-4) and 1 pRBCu (range 0-6).

Median number of days with BT > 38°C was 4 in both groups.

B. Infectious episodes after transplantation

Sepsis was documented in 3 out of 11 patients (27.27%) in both groups. Viral infections, related to adenovirus (2 patients) and CMV (1 patient), were observed in 3 out of 11 patients (27.27% only in A group (p=0.06).

C. Response to transplantation

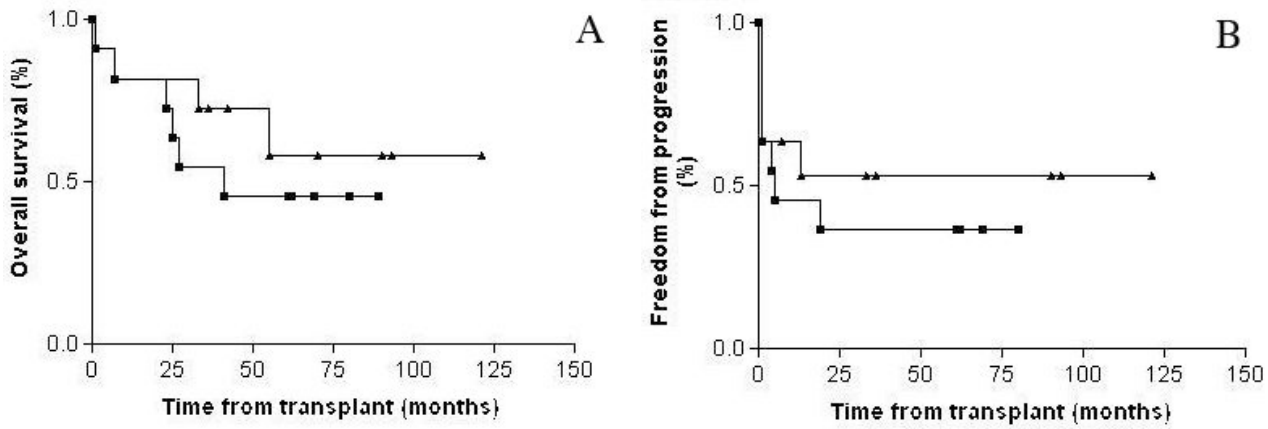
After transplant there were 7 out of 11 patients in CR (63.6%) in both groups; 4 patients (36.4%) in group A and 3 patients in group B were in PD (27.3%), 1 patient in Group B was in PR (9%).

Five patients (45%) were submitted to complementary radiotherapy after transplantation in group A while only one patient (9%) received radiotherapy in group B.

In group A, 3 patients relapsed after transplantation (42.85%) at 4, 5 and 19 months respectively. Relapse involved previous sites of the disease but unexpectedly also liver and lung in all patients. In group B, one relapse was observed (14.28%) in previous sites of the disease at 13 months. Treatment at relapse included further chemotherapy with or without radiotherapy, non-myeloablative allogeneic stem cell transplantation etc. At the time of this analysis 5 out of 11 patients in group A and 7 out of 11 patients of and group B are alive with a median follow-up of 69 months (range 61-89) and 70 (range 33-121) respectively. Four patients are in CR and 1 patient is in relapse after $CD34^+$ ASCT, whilst 6 patients are in CR and 1 patient is in relapse after unselected-PBSCT. Six patients died in group A and 4 patients in group B at a median of 24 months (range 1-41) and 20 months (range 1-55) respectively after transplantation. Median survival for patients in PD after transplantation was 16 months (range 1-27) in group A and 7 months (range 1-55) in group B respectively and was significantly shorter only for patients in PD receiving $CD34^+$ PBSCT compared to responding patients (p=0.004). Death was related to Hodgkin's disease in all patients. Survival analysis resulted in an overall survival (OS) rate of 45.5 % at a median observation time of 70 months from diagnosis in A group and 71.6 % at 70 months in B group (p=ns). Overall survival rates calculated from transplantation were 45.5% after 41 months in A group and 72.7% after 42 months in B group (p=ns) (**Figure 1A**). Disease free survival rates (calculated only for patients achieving CR after transplant) were 57.1% after a median observation time of 61 months in patients of A group and 85.7% after 70 months in B group (p=0.24). Time to progression was 5 months after $CD34^+$ ASCT with FFP of 45% and 33 months in group B with a FFP of 53% in the control group (p=ns) (**Figure 1B**).

IV. Discussion

High dose chemotherapy with autologous stem cells transplantation has been extensively used in patients with refractory or relapsing Hodgkin's disease. Data generated from single centre and from international registries clearly show that ASCT in these category of patients may produce 20-30% of long-term disease- free survivors (Hornig et al, 1997; Sweetenham, 1999). Clearly these results are superior to what salvage chemotherapy alone can now offers as shown in a randomised trial from the EBMT group for relapsed HD (Schmitz et al, 2002). Despite these encouraging results, disease relapse is still the major cause of treatment failure in the majority of patients after transplantation. One explanation may originate from the presence of neoplastic cells in the graft and also in the type of stem cell source. The presence of circulating Hodgkin/Reed-Sternberg cells of B-lymphoid origin in a patient with advanced HD has been elegantly demonstrated (Kanzler et al, 1996; Wolf et al, 1996) which were able to establish a HD derived cell line in 1997. In the mean time EBMT data showed poorer results for both progression free and overall survival in HD patients receiving PBSCT instead of bone marrow. Furthermore some studies indicated that atypical $CD30^+$ cells can be detected in the product of aphereses of Hodgkin's disease patients (Blystad et al, 2001b) and to a lesser extent in bone marrow harvest and their presence correlated with



--■--= patients submitted to CD34+ PBSC;

--▲--= patients submitted to unmanipulated PBSC.

Figure 1. Overall survival analysis (**A**) and freedom from progression analysis (**B**).

relapse after transplantation (Sharp et al, 1991; Wolf et al, 1996; Blystad et al, 2001a). A clinical benefit of removing such unwanted cells from the grafts was then suggested in the treatment of high risk patients. Then, considering the lack of CD34 antigen on the surface of Hodgkin/Reed-Sternberg cells, positive selection of $CD34^+$ cells has been applied (Blystad et al, 2001a; Lakota et al, 2002) although the significance of circulating $CD30^+$ cells is still not clear (Sharp et al, 1999).

In this study we report the results of a pilot trial carried out at our institution for patients with refractory/relapsing HD using $CD34^+$ selected peripheral blood progenitor cells. The rationale of the study was to reduce the incidence of relapse in advanced stage HD patients undergoing high dose chemotherapy and ASCT. The issue of $CD30^+$ cell contamination in the graft was not addressed in this study. Patients included in the study were then compared to a group of patients closely matched for demographic and clinical parameters and receiving unmanipulated PBPC.

Our results showed no benefit in any of the clinical endpoints in patients receiving $CD34^+$ cells. In particular, with the exception of a faster platelets recovery > 20x 10^9/l after reinfusion of PBSC no difference was observed in haematological recovery between the two groups. No differences were also found in terms of days with fever, days of i.v. antibiotics, length of hospitalization. Cautiously, the incidence of viral infections, mostly CMV infections, was felt to be slightly increased in patients receiving $CD34^+$ selected cells (Salutari et al, 1998). Despite the high efficiency of purging procedure, no evidence of benefit on CR rate, DFS or FFP was detected in patients transplanted with $CD34^+$ cells with nearly a half of the patients relapsing at a median of 5 months. Overall survival was clearly superimposable in both groups and was very prolonged with a median of 20 and 24 months respectively. These data are likely to be influenced by the management of relapse after transplantation with further chemotherapy and or local radiotherapy producing symptomatic responses and they are similar to those recently reported by other authors (Shamash et al, 2000; Paltiel et al, 2003). Disease progression produced significantly lower survival after $CD34^+$ ASCT. Interestingly relapse after $CD34^+$ transplantation involved lung parenchyma in all patients as a new site and liver in 1 patient. This behaviour also has been described by Shamash et al in up to 53% of patients with lung recurrences after unselected PBSCT (Shamash et al, 2000).

Two clinical reports were recently published using purging procedures in Hodgkin's disease (Blystad et al, 2001a; Lakota et al, 2002). In both studies $CD34^+$ selection was adopted. The first study included 10 patients with advanced HD in 2nd or higher CR at transplantation. The authors reported a strikingly high response rate with 90% of patients in CR at a median follow-up of 26 months (Lakota et al, 2002). The second study included 21 patients with HD, the majority of whom were in 2nd PR, undergoing $CD34^+$ PBSCT. In this study data were compared to a group of patients treated with unselected PBSCT. Although there was no difference between the 2 groups, EFS was 75% at 4 years in patients receiving $CD34^+$ ASCT (Blystad et al, 2001a). These differences in treatment outcome are probably related to the small number of patients treated in these series, including our own study, the length of follow up and heterogeneity of clinical characteristics and particularly status at transplantation which still remains one of the mayor determinant for the outcome of ASCT (Constans et al, 2003).

Although our data confirm that $CD34^+$ ASCT in high risk HD is feasible and safe, clinical outcome after an appropriate follow up was unaffected by the purging procedure and despite the small number of patients in our series, our results indicated that $CD34^+$ selection may not be efficient in the setting of HD at least in patients with relapsed/refractory disease.

Acknowledgements

This work was supported in part by Associazione Italiana per la Ricerca sul Cancro (AIRC) Milan, Italy. We are grateful to the nursing staff of the Divisione di Ematologia, PoliclinicoA. Gemelli.

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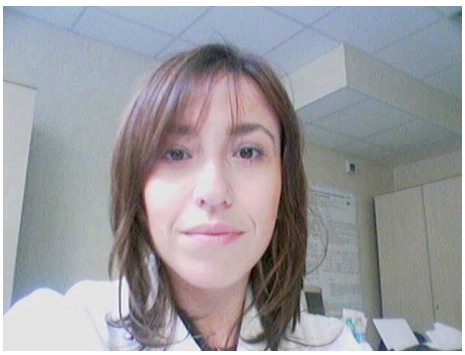

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