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ORIGINAL ARTICLE

Isolation of purified autologous peripheral blood CD34+ cells with low T cell content using CliniMACS device – a local experience

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Abstract

Introduction: Peripheral blood stem cells (PBSC) mobilised with growth factor with or without chemotherapeutic regimens, are used increasingly in both autologous and allogeneic transplantation. Previously, many PBSC harvests are used directly without *ex vivo* manipulation, and these PBSC have been shown to be contaminated with tumour cells, which may contribute to subsequent relapses post transplantation. Therefore, requirement for purging of malignant cells from the harvest has initiated the use of various methods to reduce tumour cell contamination of the graft by the positive selection of CD34+ progenitor cells or negative selection of tumour cells using other cell-specific antigens. We report here our local experience with the CliniMACS (magnetic-activated cell separation system) in eight adult patients with haematologic malignancies. *Objective:* To evaluate the purity, recovery and viability of CD34+ cells selected from harvested peripheral blood stem cells using the CliniMACS device, as well as to evaluate the T and B cell contents of these products. *Method:* Eight adult patients with malignant haematological diseases (5 non-Hodgkin's lymphomas in 2nd complete remission (CR) and 3 acute myeloid leukaemias in 1st CR) were mobilised with granulocyte colony-stimulating factor (G-CSF) with or without chemotherapeutic regimens. A total of nine leukaphereses for peripheral blood stem cell harvest using the Cobe Spectra cell separator (Cobe BCT Lakewood, CO) were performed. The harvested PBSC were then positively selected for CD34+ cells using the CliniMACS device (Milteny Biotech, Germany). *Results:* A total of nine leukapheresis products from eight adults with a median pre-selection total CD34+ cell count of 282.2 x 10^6 (range 103.7 – 738.2 x 10^6) were positively selected with CliniMACS. The median post-selection total CD34+ cell count was 99.5 x 10^6 (range 7.7 – 443.9 x 10^6) with the median recovery was 66.0% (range 2 – 94%) and median purity of products of 79% (range 18 - 86%). The median total T cell count was reduced dramatically from 3.1 x 10^9 pre-selection to 7.9 x 10^6 post-selection. The selection did not affect the viability of selected cells that was tested with trypan blue exclusion method with a median pre and post selection viabilities of 98% (range 95 - 98%). *Conclusion:* We conclude that positive selection of CD34+ cells using magnetic separation technology by CliniMACS device results in low T-cell content stem cell with acceptable purity and recovery for autologous peripheral blood stem cell transplantation.

Keywords: Autologous peripheral blood CD34+ cells, low T cell content, CliniMACS

INTRODUCTION

Peripheral blood stem cells (PBSCs) have been used increasingly as an alternative source of autologous haematopoietic stem cells replacing bone marrow to support high dose chemotherapy for a variety of malignancies. PBSCs were mobilised for autologous PBSC transplant using growth factor with or without chemotherapeutic regimens. A limitation of this technique is the contamination of the stem cell products with residual malignant cells as reported in multiple myeloma, low grade lymphoma and breast

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cancer.[1,2,3] Various purging strategies have been investigated to eliminate the malignant cells from autografts. One of the approaches is the positive selection of CD34+ haemopoietic stem cells using monoclonal antibodies to CD34 antigen, which is present on the surface of haematopoietic stem cells. Clinical studies.[4,5] have shown that this approach results in a 2-5 log reduction of contaminating tumour cells in patients with multiple myeloma, low grade lymphoma and breast cancer.

There are few devices currently available for clinical scale CD34+ selection of PBSC apheresis products; CliniMACS (Miltenyi Biotec GmbH, Bergish Gladbach, Germany) utilises a monoclonal anti-CD34 antibody, directly conjugated to an iron-oxide/dextran particle approximately 50 nm in size which binds CD34+ cells which are captured using high-gradient magnetic fields created by placing a column of small ferromagnetic beads between the poles of rare earth magnets.

We report here our evaluation of CliniMACS on purity, recovery and viability of the CD34+ cells of nine apheresed PBSCs from eight patients for autologous stem cell transplantation.

METHODOLOGY

Patients studied and PBSCs collection

In the year of June 2003 to June 2005, a total of eight adult patients were studied. Five patients with non-Hodgkin's lymphoma in 2nd complete remission (CR); of which four were mobilised with ICE (ifosfamide, carboplatin and etoposide) and granuolocyte-stimulating factor (G-CSF), and the other one was mobilised with ICE (ifosfamide, carboplatin and etoposide) and G-CSF for the first mobilisation and G-CSF only for the subsequent occasion. Three patients with acute myeloid leukaemia in first CR were mobilised with Daunorubicin and Cytarabine arabinoside plus G-CSF. Aphereses were performed on two successive days using COBE Spectra (Cobe Laboratories, Gloucester, UK) to achieve the targeted CD34+ cell counts of $\geq 2.0 \times 10^6$/kg recipient body weight.

Positive Selection of CD34+ cells

The aphereses product of the first day was diluted in equal volume of autologous plasma and stored overnight at room temperature (20 – 25 ^{o}C) in accordance with CliniMACS recommendation for harvest storage, with the cell density not exceeding 2×10^8/ml. On the next day, it was pooled with the second aphereses product, the resultant combined aphereses product was mixed, and then diluted with buffer supplemented with human serum albumin (HSA) and volume adjusted to the optimal labelling volume with total nucleated cells of $\leq 60 \times 10^9$ for each selection. The aphereses products were incubated with CliniMACS CD34 (murine anti-human CD34 antibody) for 30 minutes at room temperature and washed twice to remove unbound antibody. Prior to the selection, samples were taken for CD34+ cell enumeration, T and B cell enumeration and viability study; the labelled cells were then loaded onto the CliniMACS ® column. For labelling of the CD34 positive cells, the leukapheresis product was incubated with the CliniMACS CD34 Reagent consisting of super-paramagnetic iron-dextran particles directly conjugated to CD34 antibody (QBEND/10). After washing away the excess unbound reagent the automated selection was started. The CliniMACS system passed the antibody-lableled suspension through a column in which strong magnetic gradients were generated. The column retained the magnetically labelled CD34 positive cells, while unwanted cells flowed through and were collected in the negative fraction bag. The system performed several washing steps disposing most of the liquid into the buffer waste bag. The selected CD34 positive cells were released from the column by removing the column from the magnetic field and eluting the cells into the cell collection bag and samples of these post selection products were sent for CD34+ cell enumeration, T and B cell enumeration and viability study.

Flow cytometry

Flow cytometric analysis of CD34+ cells, enumeration of T and B cells was performed by FACScan (Becton Dickinson, USA). CD34+ cell counts of the harvested and purified products' were determined using Becton Dickinson Procount Progenitor Cell Enumeration Kit (Becton Dickinson, San Jose, CA, USA) which consists of phycoerythrin (PE)-conjugated anti-CD34, peridine chlorophyll protein (Per-CP)-conjugated anti-CD45 and a nucleic acid dye (DNA/RNA-specific dye). Harvested and purified products' T and B cell counts were also determined using a three colour flow cytometric method.

Viability measurement by trypan blue exclusion staining

The trypan blue stock solution (0.4%) (Sigma Chemical Co, Germany) was diluted 1:10 with

0.9% NaCl. Samples of the aphereses products (sample II & III) just before and after CD34+ selection were analysed. The cell suspension was mixed with 0.04% trypan blue solution at a ratio of 1:5. In a Neubauer chamber, at least 100 cells were microscopically analysed for viability in duplicate. The mean % of living cells of the two analyses was calculated.

RESULT

Purified product CD34+ cell recovery, purity and viability

A total of nine apheresis products from eight adult patients were processed for the positive CD34+ selection using CliniMACS. The median total nucleated cell count was 32 x 10^9 (range 17 – 51 x 10^9) and the median pre-selection total CD34+ cell count of the aphereses products was 282.2 x 10^6 (range 103.7 – 738.2 x 10^6); Following the process of CD34+ selection, the products showed a median CD34+ cell count of 99.5 x 10^6 (range 7.7 – 443.9 x 10^6) with the median purity of products of 79% (range 18 - 86%). The median recovery was 66.0% (range 2 – 94%). Of these cases, case 6, 7, 8 showed recovery rates of less than 50%. The manufacturer's guidelines for maximum capture of target CD34+ cells of 6 x 10^8 on CliniMACS device was exceeded in case 7. In the other two cases (case 6 and 9), the pre-selection CD34+ cell counts were less than the maximum capture capability of the antibody, but the CD34+ cell yields were 12 and 2% respectively. Besides, the product purity of cases 6 and 8 were also less than 50% (46% and 18% respectively). There was no technical or procedural error identified.

The selection did not affect the viability of selected cells that was tested with trypan blue exclusion method with a median pre and post selection viabilities of 98%. (range 94 - 99% pre selection and 92 - 99% post selection).

Purified product T and B cell content

Of the nine positively selected stem cell products, seven were analysed for the T and B cells content before and after the CD34+ selection. The T cells (CD45+/CD3+) and B cells (CD45+/CD19+) content of the pre-purified products were much higher compared to the purified products as shown in Table 2. The median total T cells content of the pre and post CD34+ selection products were 3.1 x 10^9 (range 1.0 x 10^9 – 14.0 x 10^9) and 7.9 x 10^6 (range 1.6 x 10^6 – 3.4 x 10^7) respectively. The median B cells content of the pre and post CD34+ selection PBSC products were 7.9 x 10^8 (range 0 – 1.5 x 10^9) and 3.2 x 10^5 (range 0 – 3.5 x 10^7). Analysis using the paired sample T-tests showed that the post selected stem cell products have significantly lower T cell content with the mean ± SD of T cell contents in the pre and post selected PBSC products of 523.9 ± 463.4 x 10^7 and 1.02 ± 0.78 x10^7 respectively ($p = 0.02$); whereas the B cell contents were not significantly different with the mean ± SD of pre and post selected PBSC products of 412.0 ± 620.5 x10^6 and 7.2 ± 13.0 x10^6 respectively ($p = 0.13$).

Case 8 had shown very high contamination of T-cells in the purified products (77.3%) which could explain the low product purity of 18%. Owing to the low recovery rate and product purity of cases 6, 7 and 8, we looked into the other aspect of the procedures which include the CliniMACS murine anti-human CD34 antibody as well as the selection sets. All the cases were using the same selection set except for case 9. The CliniMACS CD34 antibodies that were used for the selections were from two different lot numbers; however, investigations from the manufacturer did not yield any significant feedback.

DISCUSSION

Studies performed in the early 1990s demonstrated that peripheral blood leukapheresis autografts from patients with multiple myeloma, low grade lymphomas and breast cancer were contaminated with significant numbers of tumour cells.[1,2,3] The used of tumour cells contaminated autografts correlated with poor transplant outcomes.[6,7,8,9.] Various methods have been used to reduce the tumour cells from the autograft and one of the approaches are to positively select the CD34+ haemopoietic stem cells using monoclonal anti-human CD34+ antibodies prior to the PBSC cryopreservation with the aim to reduce the tumour burden of the graft and possible decrease the relapse rate.[3]

In the mid 90's, Lemoli RM et al[3] used the avidin-biotin immunabsorption technique to enrich PB CD34+ cells of myeloma patients achieving a median purity of 89.5% (51-94%) and median recovery of CD34+ cells of 58% (33% - 95%) thus resulting in 2.5 – 3 log depletion of plasma cells and CD19+ B cells. Following that, the increasing demand for enrichment of CD34+ cells and depletion of unwanted tumour cells from the graft, led to the development of

TABLE 1: Viability, total CD34+ cell count, percentage of CD34+ cell recovery and purity of stem cell products before and after CD34+ selection with CliniMACS device

Case number	Viability		Total CD34+ count x10^6		Recovery of CD34+ count (%)	Purity of products (%)
	Pre-selection	Post-selection	Pre-selection	Post-selection		
1	98	98	282.2	265.7	94	84
2	98	98	312.3	214.8	69	75
3	99	98	155.9	92.5	59	83
4	98	97	644.7	443.9	69	86
5	98	98	119.8	79.3	66	51
6	98	98	103.7	12.0	12	46
7	99	99	738.2	332.0	45	79
8	99	97	400.6	7.7	2	18
9	97	94	125.3	99.5	79	83
Median	**98**	**98**	**282.2**	**99.5**	**66**	**79**

TABLE 2: T and B cells content of pre and post purified products

Case number	Total CD45+ / CD3+ cell count x10^7		Total CD45+ / CD19+ cell count x10^6	
	Pre-selection	Post-selection	Pre-selection	Post-selection
1	820	0.79	1500	12
2	170	0.46	145	35
3	1400	0.35	0	0
5	660	2.0	7.9	2.7
7	100	1.4	0.98	0.08
8	120	3.4	0.23	0
9	310	0.16	130	0.32
Median	310	0.79	7.9	0.32
Mean ± SD	532.9± 463.4	1.02 ± 0.78	412.0 ± 620.5	7.2 ± 13.0
P value	p = 0.023		p = 0.135	

immunomagnetic cell selection system such as Isolex 300i (Baxter Immunotherapy Division, Unterschleissheim, Germany), CliniMACS (Miltenyi Biotech) and Ceprate SC (Cellpro).

Based on the published studies analysing the efficacies of various magnetic-based cell selection system, the documented median purity of CD34+ cells ranged from 90% to 97.7% and the median recovery were ranged from 52.3% to 97.4%. Table 3 summarises the results of various studies using the CliniMACS system.

Comparing our results with other studies that used the CliniMACS, our median recovery rate of 66% is comparable with the recovery rates of previous studies with 3/9 cases showing recovery rates of less than 50%. Case 7 had a total CD34+ cell count that exceeded the maximum capture capability of the antibody resulting in a recovery rate of 45%, and no technical error was identified in the other 2 cases (case 6 and 8). However, our median purity of 79% was slightly lower compared to those reported by previous authors, with 2/9 cases (case 6 & 8) showing purity of less than 50%. Analysis of these cases showed that case 8 was contaminated with 77.3% of T-cells and only 18% of CD34+ cells in the purified products which resulted in a very low recovery rate of 2%. Another possible reason for the low

TABLE 3: Comparison of studies using CliniMACS system for CD34+ selection

Authors	Patient group	Median purity % (range)	Median recovery % (range)
Schumm M, et al.[10] 1999	Malignant diseases	97 (68.3 - 99.7)	71 (24 – 105)
DJ Rachel, et al.[11] 2000	Breast cancer	96.1 (27.4 – 99.4)	52.3 (15.2 – 146.3)
Després D, et al.[12] 2000	Lymphomas, CLL, Multiple myeloma, ALL, medulloblastoma	97.7 (89.4 – 99.8)	69.5 (46.9 – 87.3)
Polouckova A, et al.[13] 2001	Haematological diseases, healthy donors	Allogeneic 94.8 (69.1 – 99) Allogeneic 58 (30 - 79.6)	Autologous 94 (94 – 94) Autologous 97.4 (95 – 99.8)
Michael JW et al.[14] 2002	MM, NHL, healthy donors	90 (79 – 99)	64 (24 – 76)
Prince et al.[15] 2002	Breast cancers	93 (76 – 98)	62 (16 – 93)
Leong et al. 2008	Lymphomas, AML	79 (18 - 86)	66 (2 - 94)

recovery and purity of these products is the quality of the monoclonal antibodies used. In our retrospective analysis of the CliniMACS CD34 reagents and CliniMACS tubing sets used, all the tubing sets used were of the same lot number except case 9. However, the CliniMACS CD34 antibodies that were used for the selections were from two different lot numbers, but investigations by the manufacturer did not yield any significant results. The other issue that we thought could have contributed to this was the preanalytical factors involved in the transportation of the monoclonal antibodies especially the storage temperature during the transportation, but we were unable to rectify this.

Conclusion

Positive selection of CD34+ cells using magnetic separation technology by CliniMACS device results in low T-cell content stem cell with acceptable purity and recovery for autologous peripheral blood stem cell transplantation.

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