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ORIGINAL ARTICLE

Experience of Peripheral Blood CD34+ Stem Cells Collection in Autoimmune Patients

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SUMMARY

Background: **Autologous Hematopoietic Stem Cell Transplantation with or without CD34+ selection is being used successfully to treat patients with severe and refractory autoimmune disease. This study describes our experience of CD34+ stem cell mobilization, harvesting and selection in autoimmune patients based on conditions in Vietnam - the developing country.**

Methods: **Eight autoimmune patients (four patients with Myasthenia Gravis and four patients with Systemic Lupus Erythematosus) underwent PBSC mobilization with granulocyte colony-stimulating factor (G-CSF) and cyclophosphamide. The apheresis was performed on a Terumo BCT Spectra Optia machine. CD34+ hematopoetic stem cells were collected from the leukapheresis by CliniMACS Plus device using CD34 Enrichment KIT. CD34+ cells, T and B lymphocytes were counted on a FACS BD Canto II device.**

Results: **Eight patients (4 MG and 4 SLE) including 5 females and 3 males were involved in this study. The mean age of the patients was 33.13 ± 16.64 years (ranging from 13 to 58 years). The average number of days for mobilization was 7.9 ± 1.6 days, whereas the average number of days for harvesting was 1.5 ± 0.5 days. There was no difference in the number of days for mobilization and harvesting between the MG and SLE groups. The number of CD34+ cells in peripheral blood (PB) on the day of harvesting was 108.37 ± 59.64 x 10^6 cells/L. There was a significant difference in white blood cell (WBC), neutrophil, monocyte, and platelet cell counts between before and after mobilization. On the day of stem cell harvesting, variables such as WBC, neutrophil, lymphocyte, monocyte, platelet, CD34+ cell counts, and hemoglobin were not different between the MG and SLE groups. The CD34+ recovery percentage following the CD34+ selection procedure was 68.8%, whereas almost 99.9% of the T and B lymphocytes, and NK cells in the PBSC products were eliminated.**

Conclusions: **Very first attempts in mobilizing, harvesting, and selecting CD34+ stem cells were successful, paving the way for autoimmune patients to have autologous hematopoietic stem cell transplantation in Vietnam.**
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KEYWORDS

autologous hematopoietic stem cell transplant, CD34+, ClinMACS, autoimmune diseases, Myasthenia Gravis, Systemic Lupus Erythematosus

LIST OF ABBREVIATIONS

HSCT - Hematopoietic Stem Cell Transplant
PBSC - Peripheral Blood Stem Cell
HSC - Hematopoietic Stem Cell
PB - Peripheral Blood
MG - Myasthenia Gravis
SLE - Systemic Lupus Erythematosus
TNC - Total Nucleated Cell
WBC - White Blood Cell
HGB - Hemoglobin
PLT - Platelet
SD - Standard deviation
CD - Cluster of Differentiation
g/L - gram/Liter
µg/L - microgam/Liter
mL - milliliter
µL - microliter

INTRODUCTION

Over the last 25 years, hematopoietic stem cell transplantation (HSCT) following high-dose chemotherapy has been increasingly used to treat autoimmune diseases [1,2]. Self-activated T and B lymphocytes are regarded as crucial to the pathophysiology of autoimmune disorders [3]. To reset the immune system with a diversification of the T cell receptor repertoire and functional renewal of the regulatory T and B cell compartment, achieving long-term remission, and preventing disease recurrence, HSCT with stem cell products that eliminate these abnormal cells could be a lifesaver for patients with autoimmune disorders refractory to conventional therapy [1]. Immunomagnetic enrichment of CD34+ hematopoietic stem cells (HSC) using paramagnetic nanobead linked CD34 antibody and immunomagnetic extraction with the CliniMACS Plus system is the standard method for producing T-cell-depleted stem cell products [4]. Even though this approach has been utilized in many parts of the world over the past two decades, it is still a rarity in Vietnam. The aim of this study was to describe our experience of CD34+ stem cell mobilization, harvesting, and selection in autoimmune patients, the very first experience in our country.

MATERIALS AND METHODS

Subjects and PBSC collection

All methods and experiments in this study were conducted in accordance with the ethical standards outlined in the Helsinki Declaration of 1975, as revised in 2008, as well as national law. In addition, the study was approved by the Hanoi Medical University's Ethics Committee for Biomedical Research (IRB00003121, approval number 87/GCN-HDDDNCYSH-DHYHN, date 12/6/2020). All patients who participated in the study provided their informed consent.

Between 12/2020 and 12/2022, eight autoimmune patients (four patients with MG and four patients with SLE) received autologous hematopoietic stem cell transplantation to treat their disease. The median patient age was 34 years (range 13 - 58), and the female-to-male ratio is 5/3. The median patient body weight was 53.5 kg. All patients underwent PBSC mobilization with cyclophosphamide 2 g/m^2 on day 1 and G-CSF 10 µg/kg on day 4 until CD34+ cell counts in peripheral blood reached > 10 cells/microliter. Apheresis was subsequently performed utilizing the Spectra Optia system - Terumo BCT in order to achieve the desired CD34+ cell counts of 2.0 x 10^6/kg patient body weight. Before CD34+ selection, leukapheresis materials were kept overnight at 2 - 8°C in a refrigerator.

Positive Selection of CD34+ cells

CD34+ cells were immunoselected from the leukapheresis products according to a standard Miltenyi protocol by CliniMACS® plus Instrument [5]. The procedure employs these following reagents and consumables: CliniMACS® CD34 Reagent (consists of a murine IgG1 monoclonal antibody directed human CD34 antigen), CliniMACS® Tubing Set TS, CliniMACS® PBS/EDTA Buffer, Human serum Albumin (HAS) 5%.

The apheresis products were incubated for 30 minutes at room temperature with CliniMACS CD34 (murine anti-human CD34 antibody) and washed twice to eliminate unbound antibodies. Before selection, samples were taken for CD34+ cell counting, T and B cell counting, and viability analysis; the labeled cells were then loaded onto the CliniMACS® column. Leukapheresis product was incubated with the CliniMACS CD34 Reagent, which consists of super-paramagnetic iron-dextran particles directly conjugated to CD34 antibody, for tagging CD34-positive cells. After removing excess unbound reagent, the automated selection process was initiated. The CliniMACS device passed the antibody-labeled suspension through a magnetic gradient-generating column. Magnetically labeled CD34 positive cells were trapped by the column, whereas non-target cells passed through and were collected in the negative fraction bag. The system performed multiple washing steps, with the majority of the liquid being disposed of in the buffer waste bag. The post-selection products were sent for CD34+ cell enumeration, T and B cell enumeration, and viability assessment.

CD34+ cell, T and B cell content enumeration

Using a flow cytometer, the total number of CD34+, CD3+, and CD19+ cells, the recovery and viability of CD34+ cells, and CD3+ depletion of leukopheresis and

post-selected products were determined (Facs Canto II Flowcytometry and DIVA software), with 7-AAD viability dye added. The percentage of 7-AAD-negative cells in a population of CD34+ cells was used to evaluate the viability of the cells. A hematology analyzer was used to measure the white blood cell (WBC) count with differential, hemoglobin (HGB), and platelets (Advia 2120i, Siemens). The recovery of viable CD34+ cells and the depletion of CD3+ and CD19+ cells were calculated as the ratio of the absolute viable CD34+, CD3+, and CD19+ cell number in the post-selected product to the absolute viable CD34+ cell number in the leukapheresis.

Microbiological cultures

Each 0.5 mL sample of leukaphereis and post-selected products is used for testing of potential microbial contamination.

Statistical methods

Statistical analysis was conducted using SPSS22.0 software (IBM, USA). The paired-samples *t*-test was used to calculate group comparisons. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Patients' characteristics

Eight patients (4 SLE and 4 MG) with a mean age of 33.13 ± 16.64 years were included in the study (ranging from 13 to 58 years). There were 5 women (representing 62.5% of the patients) and 3 men (representing 37.5% of the patients). MG patients included 3 female patients and 1 male patient. Two female and two male patients were included in the SLE group.

Characteristics of PBSC collection

All patients were successfully mobilized with stem cells to achieve the target of > 2 x 10^6 CD34+ cells/kg of body weight. The average number of days of mobilization (from the date of cyclophosphamide administration) was 7.9 ± 1.6 days, and the average number of days of harvesting was 1.5 ± 0.5 days. There was no difference between the MG and SLE groups in the number of days of CD34+ stem cell mobilization (8.5 days and 7.3 days, respectively, p = 0.08). All SLE patients required stem cell harvesting in 2 days, whereas 3 MG patients required harvesting within 1 day and 1 patient required harvesting in 2 days. The difference was not statistically significant, though.

Parameters before mobilization and apheresis day

Table 1 shows the white blood cell, neutrophil, lymphocyte, monocyte, hemoglobin, and platelet counts on the day preceding mobilization therapy administration and the day of collection. WBC, neutrophil, and monocyte counts, as well as platelet counts, display statistically significant differences, whereas HGB and lymphocyte counts do not. The average number of CD34+ cells in peripheral blood on the day of collection was 108.37 ± 59.64 cells/L, and the survival rate was 99.01 ± 0.88 percent.

Parameters of apheresis day in MG and SLE group

Peripheral blood parameters on the day of stem cell harvest revealed no statistically significant differences between MG and SLE patients in terms of white blood cell count, neutrophil count, lymphocyte count, monocyte count, hemoglobin level, and platelet count.

Properties of PBSC product before and post CD34+ selection

The CD34+ stem cell selection procedure obtained a recovery efficiency of 68.8% for CD34+ cells and removed over 99.9% of T, B, and NK cells. There was no difference in the percentage of CD34+ cells that survived before and after the process, and no microbial infection occurred during the procedure (Table 3).

DISCUSSION

This study addresses the mobilization, harvesting, and selection of CD34+ stem cells in an autoimmune patient receiving hematopoietic stem cell transplantation. In the last two decades, over 3,000 autoimmune patients have been treated with hematopoietic stem cell transplantation, primarily autologous hematopoietic stem cell transplantation [1,2]; nonetheless, these are the first patients in Vietnam to experience this therapy. The quality of the stem cell products used for hematopoietic stem cell transplantation plays a crucial role in determining the efficacy of the treatment; therefore, procedures to mobilize, collect, and select CD34+ stem cells in each center must be evaluated. In the two years between 12/2020 and 12/2022, our laboratory center mobilized and collected stem cells from four patients with Myasthenia Gravis and four patients with Systemic Lupus Erythematosus, with a mean age of 33.13 ± 16.64 years. Three of the four patients with Myasthenia Gravis were women with postpartum onset, and one was a 58-year-old man. This is consistent with previous research on the age of onset of Myasthenia Gravis in women and men, which indicate that women typically develop Myasthenia Gravis during childbearing age, while men commonly develop Myasthenia Gravis older with an average age of onset over 50 years old [6]. There were two males and two females among the four Systemic Lupus Erythematosus patients. Because our sample size is limited, this rate does not correspond to the gender ratio among Systemic Lupus Erythematosus patients.

To mobilize hematopoietic stem cells from bone marrow to peripheral blood, all patients received cyclophosphamide at a dose of 2 g/m^2 of skin on day 1, followed by G-CSF at a dose of 10 µg/kg three days later, with a mean mobilization time of 7.9 ± 1.6 days (6 to 10 days after cyclophosphamide administration) until peripheral

Table 1. Parameters of pre-mobilization and apheresis.

Patients (n = 8)			
Parameters	**Pre-mobilization (mean ± SD)**	**Apheresis (mean ± SD)**	**p-value**
WBC (x 10^9 cells/L)	5.99 ± 2.32	17.01 ± 10.08	0.024
Neutrophil (x 10^9 cells/L)	3.93 ± 1.54	14.74 ± 9.59	0.025
Lymphocytes (x 10^9 cells/L)	1.28 ± 0.55	0.49 ± 0.36	0.081
Monocytes (x 10^9 cells/L)	0.45 ± 0.17	0.97 ± 0.34	0.021
HGB (g/L)	134.1 ± 14.2	121.6 ± 13.1	0.052
Platelets (x 10^9 cells/L)	291.0 ± 36.5	135.7 ± 42.6	< 0.0001
CD34+ (cells/µL)		108.37 ± 59.64	
CD34+ viable (%)		99.01 ± 0.88	

WBC - white blood cell, HGB - hemoglobin, SD - standard deviation, CD - cluster of differentiation, L - Liter, g/L - grams/Liter.

Table 2. Parameters of apheresis in MG and SLE group.

Parameters	SLE group (n = 4) (mean ± SD)	MG group (n = 4) (mean ± SD)	p-value
WBC (x 10^9 cells/L)	18.13 ± 4.44	16.99 ± 16.67	0.919
Neutrophil (x 10^9 cells/L)	16.24 ± 4.31	14.54 ± 15.69	0.874
Lymphocytes (x 10^9 cells/L)	0.23 ± 0.26	0.54 ± 0.13	0.095
Monocytes (x 10^9 cells/L)	0.89 ± 0.12	0.95 ± 0.53	0.883
HGB (g/L)	118.0 ± 18.1	127.0 ± 10.5	0.639
Platelets (x 10^9 cells/L)	104.67 ± 20.03	142.33 ± 29.16	0.196
CD34+ (cells/µL)	92.74 ± 48.35	124.01 ± 72.87	0.499

MG - Myasthenia Gravis, SLE - Systemic Lupus Erythematosus, WBC - white blood cell, HGB - hemoglobin, SD - standard deviation, L - Liter, g/L - grams/Liter.

Table 3. Parameter of leukapheresis product and post-CD34+ selection.

Patients (n = 4)				
Parameters	**Leukapheresis product (mean ± SD)**	**Post-CD34+ selected (mean ± SD)**	**CD34+ recovery (%)**	**CD3+, CD19+ depletion (%)**
TNC (x 10^9 cells)	16.87 ± 6.56	0.32 ± 0.07		98.1
CD34+ (x 10^6 cells)	463.83 ± 106.33	356.09 ± 138.41	68.8	
CD34+ viable (%)	96.1 ± 2.8	94.8 ± 3.1		
CD3+ (x 10^6 cells)	3268.16 ± 1225.94	0.08 ± 0.05		99.99
CD19+ (x 10^6 cells)	305.64 ± 138.54	0.13 ± 0.12		99.95
CD56+ (x 10^6 cells)	84.34 ± 54.08	0.005 ± 0.003		99.99
Bacterial culture	all negative	all negative		
Fungal culture	all negative	all negative		

PBSC - peripheral blood stem cell, TNC - total nucleated cell, CD - cluster of differentiation.

blood CD34+ counts exceeded 10 cells/µL. The mobilization period in our investigation is comparable to that of other studies, including those of Milone G et al. (7 - 11 days) [7] and Jaime-Perez JC et al. (7 days) [8]. All SLE patients required stem cell harvesting in 2 days, whereas 3 MG patients required harvesting within 1 day and 1 patient required harvesting in 2 days. MG is an organ-specific autoimmune disorder that only affects the neuromuscular system. SLE is a systemic autoimmune disease involving multiple organs, including the bone marrow, with features of fibrosis, pure red cell aplasia, and aplastic anemia. This may impact the mobilization of stem cells from the bone marrow into the peripheral circulation. We could not identify a statistically significant difference, however, presumably because of the limited sample size.

On the day of collection, the average number of nucleated cells was 17.01 ± 10.08 x 10^9/L, neutrophil was 14.74 ± 9.59 x 10^9/L, hemoglobin was 121.6 ± 13.1 g/L, platelet count was 135.7 ± 42.6 x 10^9/L, and the CD34+ cells count was 108.37 ± 59.64 cells/µL. Before administering chemical mobilization on the day of harvest, the amount of white blood cells, neutrophils, monocytes, and platelets was significantly different. A study by L Statkute et al. on 130 autoimmune patients who received hematopoietic stem cell transplantation revealed that the average peripheral blood white blood cell count was 31 x 10^9/L, platelets was 154 x 10^9/L, and hemoglobin was 110 g/L, and the average number of CD34+ cells on harvest day was 99 cells/µL, which is comparable to our study [9]. A study by Jaime-Perez JC et al. in 51 multiple sclerosis patients also using cyclophosphamide and G-CSF mobilization had a peripheral blood stem cell count of 51.29 (2.64 - 260.92) cells/L [8], which was lower than the observed results in our analysis.

Comparing the peripheral blood (PB) variables on the day of harvest between the 2 groups of patients with Myasthenia Gravis and patients with Lupus, we did not see a statistically significant difference. A previous meta-study by L Statkute et al., which compared these indices between groups of autoimmune patients, showed that the highest mean PB WBC/mL and PB CD34+ cells/mL were achieved in patients with multiple sclerosis (MS), the lowest were in patients with SLE and systemic sclerosis (SSc). Mean PB CD34+ cell percentage was the highest in patients with relapsing remitting MS, and lowest in patients with SSc and secondary progressive MS [9]. It is possible that because of our small sample size, this difference was not found in our study. It is assumed that autoimmune T and B lymphocytes play a key role in the etiology of autoimmune disease [3]. Theoretically, simultaneous *ex-vivo* and *in-vivo* eradication of this autoimmune cell could minimize the chance of recurrence, hence increasing the duration of patients' remission. It is true that numerous patients with autoimmune disorders have had CD34+ selective hematopoietic stem cell transplantation [1], but there is also controversy, since a recent multicenter study found that the selection of CD34+ cells does not improve the result of autologous HSCT for SSc patients. There were no significant changes in overall survival, progression-free survival (PFS), incidence of relapse or progression, between patients treated with mononuclear cells or pure CD34+ cells [10]. This observation was comparable to a previous study by Moore et al., which compared pure CD34+ cells and unmanipulated cells in autologous HSCT for the treatment of rheumatoid arthritis [11]. However, both studies recommend further evaluation. In this study, we perform CD34+ selection of PBSC products from 4 patients (2 patients with MG and 2 patients with SLE). Each patient's leukopheresis product is separated into two bags to fit into the small-scale kit we have available, which has a capacity of less than 0.6 x 10^9 CD34+ cells out of a total of 60 x 10^9 nucleated cells. Post-CD34+ selection products contained a TNC (total nucleated cell) count of 0.32 ± 0.07 x 10^6 cells, an average CD34+ cell count of 356.09 ± 138.41 x 10^6 cells with a survival rate of 94.8 ± 3.1%, and an average residual T, B, and NK (natural killer) cell count of 0.08 x 10^6, 0.13 x 10^6, and 0.005 x 10^6 cells, respectively. The median recovery of CD34+ cells is 68.8%, and the depletion of T, B, and NK cells exceeds 99.9%. None of the final stem cell products were contaminated bacteria or fungus.

The recovery of CD34+ cells in our study is comparable to those of earlier researchers such as Gabriele Spohn et al., Leong et al., Despres et al., and Imai et al., whose respective values were 72.4 ± 2.8%, 66% (2 - 94%), 69.5% (46.9 - 87.3%), and 72% [12-15]. At the same time, the capacity to remove T and B cells following a CD34+ selection procedure is comparable to that described in the preceding research.

CONCLUSION

The efficiency of HSCT with either CD34+ selection or non-selection need to be further evaluated with a larger number of patients and longer-term follow-up. However, those approaches must be promising options to address the complexity of the disorders even in developing countries such as Vietnam. Our data showed that the quality of HSC units that were mobilized and collected are comparable and reached the standard for treatment for autoimmune diseases. Furthermore, we also succeeded in selecting CD34+ with high purification and almost eliminating the activated lymphocytes. By minimizing the presence of matured blood cells, the autologous CD34+ HSCT should result in a more optimal treatment of autoimmune patients.

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Declaration of Interest:
All authors declare no conflict-of-interest regarding this article.

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