Purification of CD34+ cells in myasthenia gravis patient's peripheral blood stem cells using the CliniMACS cell separation system

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Summary

Autologous hematopoietic stem cell transplantation therapy has been widely used in recent decades for the treatment of hematological diseases, cancer, and autoimmune diseases. In autoimmune diseases, self-activated T lymphocytes and B lymphocytes secreting antibodies against host antigens are considered to be central in the pathogenesis of the disease. Autologous stem cell products that eliminates these pathological cells could be a lifesaver for patients with autoimmune diseases who are resistant to conventional therapy, in order to restore a healthy immune system, achieve long-term remission and limited recurrence. The aims of this study was to complete and evaluate the results of the CD34 positive stem cells purification procedure from a myasthenia gravis patient's mobilized peripheral blood stem cells, for the purpose of autologous hematopoietic stem cell transplantation for Myasthenia gravis as well as other autoimmune diseases in Vietnam. We have completed the purification procedure of CD34-positive stem cells from mobilized peripheral blood stem cells of patient with myasthenia gravis using CliniMACS system with results of removing 99.99% of T lymphocytes; 99.81% of B lymphocytes; 99.99% of NK cells, while CD34+ recovery performance was 64.05% with CD34+ cell survival rate of over 99%, no bacterial contamination, ensuring quality assurance of stem cell product for transplantation.

Keywords: HSCT, PBSC, CD34 selection, myasthenia gravis, CliniMACS, autoimmune disease.

1. Background

Myasthenia gravis (MG) is a chronic autoimmune disorder in which antibodies destroy the communication between nerves and muscles, resulting in weakness of the skeletal muscles. This disorder affects the voluntary muscles of the body, especially those that control the eyes, mouth, throat and limbs. A myasthenia gravis crisis can involve difficulty in swallowing and breathing [1]. Although we have not yet identified all drivers for the MG disease course, recent advances in therapy have been accomplished due to a clearer understanding of the pathophysiology of MG, including internal medicine (anticholinesterase agents, corticosteroids, new immunosuppressive agents, IVIG), surgery (thymectomy; thymoma removal) and hemodialysis, however, there is still an actual proportion of 10-

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15% of patients with refractory myasthenia gravis (unresponsive to the above mentioned treatments), facing life-threatening complications such as acute respiratory failure leading to death [2]. Therefore, more research is needed to develop advanced therapy to support conventional treatments with the aims of extending survival and improving quality of life.

Autologous hematopoietic stem cell transplantation has been used since 1996 for the treatment of refractory - severe autoimmune diseases with possitive results [3]. T cells, B cells secreting antibodies against acetylcholine receptors play a central role in the pathogenesis of myasthenia gravis [4]. Autologous hematopoietic stem cell transplantation with these auto-reactive T cells and B cells depletion can bring benefits to patients with myasthenia gravis, prevent disease recurrence. There were 10 patients with refractory myasthenia gravis who have been received purified CD34+ autologous hematopoietic stem cell transplantation over the world, with good results [10, 11, 12].

In Vietnam, this is the first case of myasthenia gravis that is applied this therapy. This report initially evaluates the efficacy of CD34+ cells purification method in peripheral blood stem cell collection of patients with myasthenia gravis.

2. Case presentation

41-year-old myasthenic gravis patient meets selection criteria for stem cell transplantation, was received chemotherapy to mobilize peripheral blood stem cells with cyclophosphamide 2g/m^2 on day 1, G-CSF 10µg/kg from day 4 to day 9. Stem cells were harvested on day 10. The patient's blood was centrifuged to separate blood components, cell collection using the TERUMO BCT Spectra Optia. After that, the peripheral blood stem cell collection were processed and selected for CD34+ cells using CD34-specific monoclonal antibodies conjugated to magnetic particles and the CliniMACS Plus cell separator system. For labeling of the CD34+ cells, the apheresis product is incubated with the CliniMACS CD34 Reagent. After washing away the excess unbound reagent, the automated selection is started. The CliniMACS Plus passes the antibody-labeled suspension through a column in which strong magnetic gradients are generated. The column retains the magnetically labeled CD34+ cells while unwanted cells flow through and are collected in the negative fraction bag. The system performs several washing steps that dispose of most of the liquid into the buffer waste bag. The selected CD34+ cells are released from the column by removing the column from the magnetic field and eluting the cells into the cell collection bag. The number of CD34+ cells in the final product was counted by flow cytometry using a Facs Canto II machine, and cell survival was assessed by 7-aminoactinomycin D (7-ADD) staining.

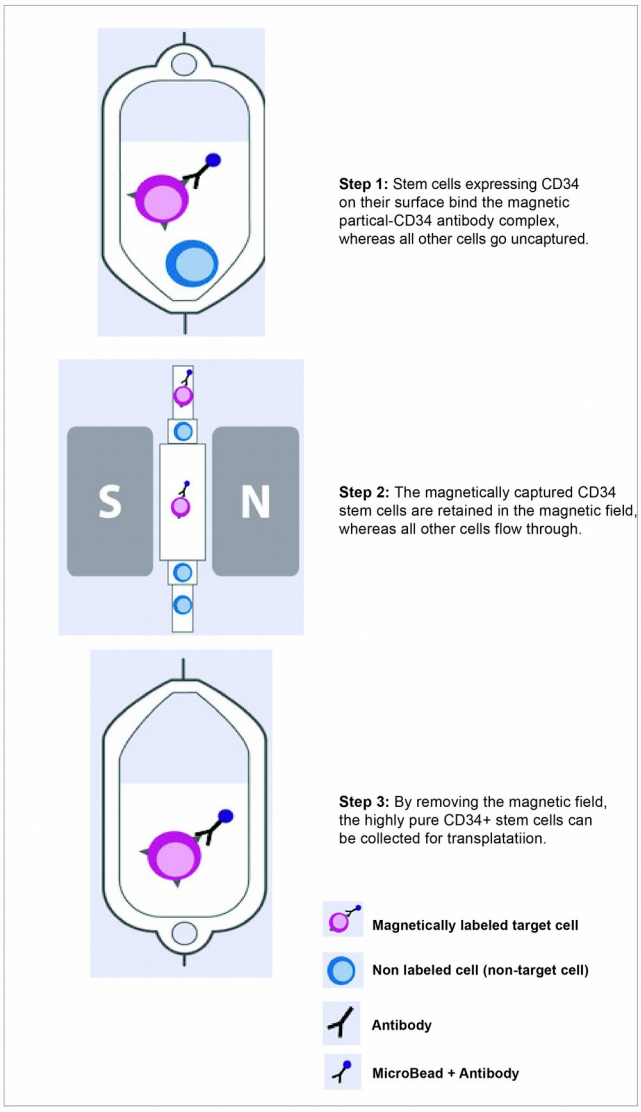

Figure 1. CD34+ cell purification procedure

The research results are as follows:

Table 1. Characteristics of apheresis products

Characteristic	Value
TNC (cells/µl)	88060.23
Total CD34+ cells (cells/µl)	6945.47
CD34+ viability (%)	99.25%
V (ml)	290ml
Time of collection	1 day
CD34+ cells dose (cells/kg)	40.28×10^6

The total CD34+ cells in apheresis product was very high, equivalent to a CD34+ cells dose of 40.28 x 10^6 cells/kg (patient's weight: 50kg).

Table 2. Characteristic of CD34+ cells purified product

Characteristics	Apheresis product	CD34+ cell purified product
Total CD34+ cells (cells)	1070.94×10^6	687.78×10^6

CD34+ viability (%)	99.14	99.12
Total lympho T (cells)	275780.72×10^4	21.82×10^4
Total lympho B (cells)	28630.16×10^4	56.26×10^4
Total NK (cells)	12630.19×10^4	1.02×10^4
CD34+ cell recovery (%)	64.05	
T cell removal efficiency (%)	99.99	
B cell removal efficiency (%)	99.81	
NK cell removal efficiency (%)	99.99	

Due to the very high amount of CD34+ cells obtained, the apheresis product was divided into 2 equal parts, one for immediate storage (for backup) and the other for CD34+ cells selection by CliniMACS.

Table 2 shows that the CD34+ cell purified procedure on the CLiniMACS system removed 99.99% of T lymphocytes, 99.81% of B lymphocytes, 99.99% of NK cells which are important cells in the pathogenesis of myasthenia gravis, while recovery most of the CD34+ stem cells with a recovery performance of 64.05%, and without significantly changing the survival rate of CD34+ cells.

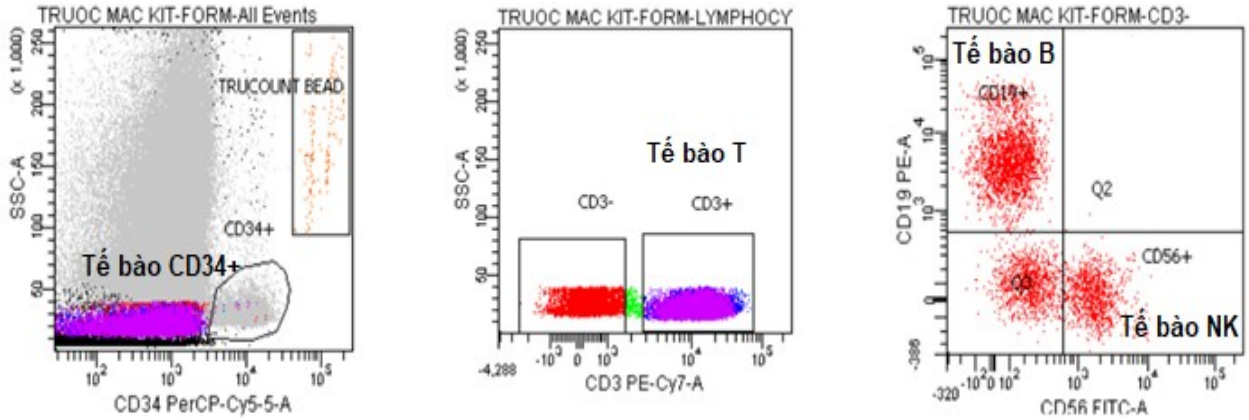

Figure 2A. CD34+ cells, T cells, B cells, and NK cells before CD34+ purification

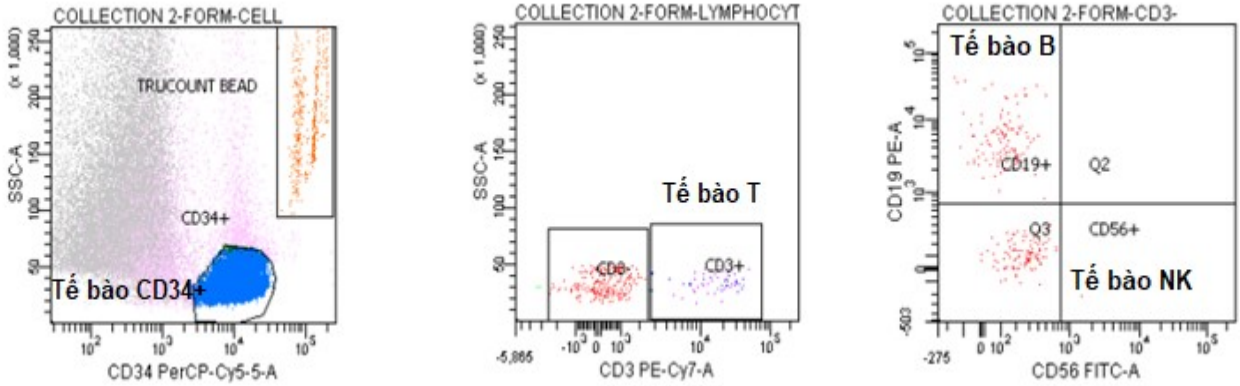

Figure 2B*: CD34+ cells, T cells, B cells, and NK cells after CD34+ purification*

Figure 2. CD34+ cells, T cells, B cells, and NK cells before and after CD34+ purified procedure

Table 3. Quality of CD34+ purified product

CD34+ cell dose (cells/kg)	**$13{,}75 \times 10^6$**
T cell dose (cells/kg)	$0{,}44 \times 10^4$

Bacterial culture	(-)

Table 3 shows that the quality of the purified CD34+ stem cell product after CD34+ cell selection meet the standard criteria with the expected dose of T lymphocytes for transplantation was only 0.44 × 10^4 cells/kg body weight. CD34+ cell dose reached 13.75 × 10^6 cells/kg and the final product was completely sterile.

3. Discussion

Hematopoietic stem cell transplantation (HSCT) has evolved over the past 20 years as a specific treatment for patients with autoimmune disease. The European Blood and Marrow Transplantation Database has accumulated more than 2000 autoimmune patients who received a hematopoietic stem cell transplant between January 1994 and December 2015, as reported by 247 centers in 40 national [5]. Stem cell source from healthy donors promises to regenerated a completely new, healthy immune system after a very intensive conditioning regimen course that is administered to destroy the auto-active cells. The stem cells from healthy donors are free of auto-immune cells but it has potential risk of life-threatening graft-versus-host disease, beside the rare supply source due to the need for HLA-matching [6]. With the advantage of always available, no risk of graft-versus-host disease, autologous hematopoietic stem cell is now the most common source of stem cells used in transplantation; However, there is also a risk of disease recurrence because the pathological cells are harvested along with the stem cells and re-infused into the patient. Especially for autoimmune diseases, the pathogenic role of B and T lymphocytes is well known [7]. Remove these pathological cells ex-vivo from stem cell products has been investigated and used in the past decade, in which ex-vivo CD34+ selection or ex-vivo T-cell removal using the ClinMASC magnetic cell separation system is a modern and commonly used technique [8, 9].

Until now, there were 10 MG patients receiving hematopoietic stem cell transplantation reported. Post-transplant results of all 10 patients were very positive with long-term remission, lower dose of immunosuppressive agents needed [10, 11, 12]. Nine patient received CD34+ selective autologous stem cell transplantation, only one patient received allogeneic stem cell transplant which was performed in 2009 [12]. In Vietnam, this is the first time that stem cell transplantation has been used in patients with myasthenia gravis, under a national - level project aimed at evaluating the effectiveness of autologous hematopoietic stem cells transplantation in the treatment of myasthenia gravis and systemic lupus erythematosus, conducted at the 108 Central Military Hospital. Our initial study aims to complete the procedure of preparing CD34+ selective stem cell product for transplantation to the patients with myasthenia gravis as well as other autoimmune diseases. The research results showed that even though this is the first patient who undergo peripheral blood stem cell mobilization, the results were more than expected, with the initial stem cell dose was as high as 40.28 × 10^6 CD34+ cells/kg body weight (Table 1).

We stored immediately ½ of the apheresis product as a backup in case the results of the CD34+ selection procedure were not as expected. The second part of apheresis product is processed to retain only CD34+ stem cells and eliminate autoimmune T cells and B cells by using monoclonal antibodies bound specific to CD34 antigen on stem cells and the CliniMACS system, the CD34+ cell recovery efficiency was 64.05%, similar to Després et al's research, the CD34+ recovery efficiency was 69.5% (ranging from 46.9% to 87.3%) [9], Imai et al conducted a research on PBSC products of 48 patients with non-Hodgkin's lymphoma showed an average CD34+ recovery rate of 72% [13]. Leong et al did a study on apheresis products from 8 patients with hematologic malignancies, recovered 66% of CD34+ cells (ranging from 2 - 94%) [14]. Regarding

the ability to remove pathological cells from the PBSC's product, our procedure removed 99.99% of T lymphocytes; 99.8% B lymphocytes; 99.99% of NK natural killer cells. While Imai's research show the purity was 93.3% (ranging from 32.6% to 99.3%) [13]; Depres's research had a T-cell removal rate of 97.7% (ranged from 89.4% to 99.8%) [9]; Lopez's study removed 99% of the unnecessary cells [15]. Leong's study had a cell purity of 79% (ranged 18% to 86%) [14]. Thus, even though it is the first time to perform CD34+ stem cell purified procedure in patient with myasthenia gravis, our results were encouraging, similar to other studies.

Assessing the quality of the final CD34+ stem cell product, we found that the product met the appropriate criteria for transplantation (Table 3), in which the CD34+ dose was very high of 13.75×10^6 cells/kg body weight, T lymphocyte dose is only 0.44×10^4 cells/kg body weight, and the final product is completely sterile after a very complicated CD34+ purification process. Most autologous hematopoietic stem cell transplants for autoimmune diseases currently require a CD34+ dose of 3-8 $\times 10^6$ cells/kg body weight and the remaining T cell count less than 2.5×10^4 cells/kg body weight [5].

4. Conclusion

The study has completed the procedure of purifying CD34+ stem cells of PBSC product in patient with myasthenia gravis using the CliniMACS system, achieving CD34+ recovery of 64.05%, removing 99.99% of T lymphocytes, 99.81% of B lymphocytes which are the pathological cells of myasthenia gravis.

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