RESEARCH ARTICLE / Araştırma Makalesi

Mobilization Success Rate Following Autologous Peripheral Blood Stem Cell Transplantation

Otolog Periferik Kan Kök Hücre Transplantasyonu Sonrası Mobilizasyon Başarı Oranı

Demet Çekdemir[1], Fergun Yilmaz[1], Fatma Ozsan[2], Nur Soyer[1], Bahar Arik[3], Sevgi Mir[2], Murat Tombuloglu[1], Ayhan Donmez[1]

[1] Department of Hematology, Ege University Medical Faculty, Izmir, Turkey
[2] Department of Pediatric Nephrology, Ege University Medical Faculty, Izmir, Turkey
[3] Apheresis responsible nurse, Department of Hematology, Ege University Medical Faculty, Izmir, Turkey

Yazışma Adresi / Correspondence:
Ayhan Donmez
Department of Hematology, Ege University Medical Faculty, 35100, Izmir, Turkey
T: **+90 232 390 35 63** E-mail: **ayhan.donmez@ege.edu.tr**

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Abstract

Aim If there is successful mobilization following a conditioning regimen for stem cell transplantation, the in vivo purged stem cell products may be collected for a second transplantation. **(Sakarya Med J 2018, 8(3):562-567)**

Methods In this prospective study, peripheral blood CD34+ cells were quantified between 6th day after stem cell infusion and 3rd day after neutrophil engraftment in autologous peripheral blood stem cell transplantation (auto-PBSCT) 16 patients using flow cytometry.

Results The CD34+ cell count was lowest during the first 10 days. The median CD34+ cell count began to increase at the 11th day and the highest value was achieved at the 14th day. We documented evidence of mobilization in only one patient (6.25%).

Conclusion Our results indicate that the rate of successful stem cell mobilization is very low following auto-PBSCT. In conclusion, collecting CD34+ stem cells following the first auto-PBSCT is not an effective method for tandem transplantation.

Keywords Antigens, CD34 (D23.050.301.264.035.134); Hematopoietic Stem Cell Mobilization (E02.095.410); Stem Cell Transplantation (E02.095.147.500.500).

Öz

Amaç Kök hücre nakli için bir koşullandırma rejiminin ardından başarılı mobilizasyon varsa, in vivo temizlenmiş kök hücre ürünleri ikinci bir nakil için toplanabilir. **(Sakarya Tıp Dergisi 2018, 8(3):562-567).**

Yöntem Bu prospektif çalışmada, periferik kan CD34 + hücreleri, kök hücre infüzyonundan 6 gün sonra ve otolog periferik kan kök hücre transplantasyonu (oto-PBSCT) 16 sitometri kullanan nötrofil engraftasyonundan 3 gün sonra ölçülmüştür.

Bulgular CD34 + hücre sayısı ilk 10 gün içinde en düşüktü. Medyan CD34 + hücre sayısı 11. günde artmaya başladı ve en yüksek değer 14. günde elde edildi. Sadece bir hastada (% 6.25) mobilizasyon kanıtını belgeledik.

Sonuç Sonuçlarımız, oto-PBSCT'yi takiben başarılı kök hücre mobilizasyon oranının çok düşük olduğunu göstermektedir. Sonuç olarak, ilk oto-PBSCT'yi takiben CD34+ kök hücrelerinin toplanması tandem transplantasyonu için etkili bir yöntem değildir.

Anahtar Kelimeler Antijenler, CD34 (D23.050.301.264.035.134); Hematopoetik Kök Hücre Mobilizasyonu (E02.095.410); Kök Hücre Transplantasyonu (E02.095.147.500.500).

Introduction

CD34+ cell levels reaching the highest levels in the first hour after infusion and decreasing to pre-infusion levels on the first day indicate that infused peripheral hematopoietic stem cells (HSCs) move away from the circulation to the tissues in hours in auto-peripheral blood stem cell transplantation (auto-PBSCT). The highest CD34+ cell levels in the first hour after infusion may reflect the effects of mixing and equilibration of infused CD34+ cells in the vascular and other (bone marrow, lung, spleen, liver) CD34+ cell compartments.[1,2]

There is limited data regarding the serum level of the hematopoietic stem cells (HSCs) after infusion in humans. In our previous study, we documented HSCs serum levels for 5 days following transplantation.[3] The level of peripheral blood CD34+ cells 6 days after the stem cell infusion might predict the relationship between the results and engraftment time. More importantly, we can document the rate of successful mobilization following neutrophil engraftment. If there is successful mobilization, these in-vivo purged stem cells may be used tandem transplantation.

We prospectively determined peripheral blood CD34+ cell counts detected consecutively between the 6th day after stem cell infusion and third day after neutrophil engraftment. We aimed to determine the success rate of mobilization following neutrophil engraftment. In addition, a possible correlation between achieved CD34+ cell levels and the engraftment times for the neutrophils and platelets were investigated.

Materials and Methods

Study Design: The study has been conducted in accordance with the principles of the Helsinki Declaration and approved by the local Institutional Review Board (09-3.1/4). This prospective study included 16 patients (M/F: 9/7) treated with auto-PBSCT. The median age of the patients was 39 (range, 24 to 62) years. The diagnosis of the patients were lymphoma (n=11), and multiple myeloma (n=5). The patients' characteristics were summarized in Table 1.

PBSC mobilization and collection: Mobilization was performed by G-CSF with (n=13) or without (n=3) chemotherapy (Table 1). G-CSF was administered in split dose twice a day. Leukapheresis was performed when the circulating CD34+ cell count was greater than 10/μL, in patients who were treated by chemotherapy and GCSF. The leukapheresis was performed on the fifth day in patients who were treated by G-CSF alone (Fresenius COMTEC, Fresenius KABI AG, Bad Homburg, Germany).

Overnight storage and PBSC cryopreservation: For auto-PBSCT, leukapheresis products were stored overnight in a commercial refrigerator without the addition of media. After an overnight storage, the leukapheresis products were diluted with autologous plasma to obtain a target NC concentration of 200x109/L. At a different location, a mixture of 15% DMSO (Hybri- Max, Sigma, St Louis, MO, USA), 6% HES (Expahes Sterile; Baxter, Turkey) and 25% autologous plasma was prepared. Diluted products and cryopreservation media were mixed at a ratio of 1:1 in bags (Cryocyte Freezing Container, 500 ml, Nexcell). The final products included 7.5% DMSO and 3% HES in bags with a NC concentration of 100x109/L, and were placed in a -80oC mechanical freezer after obtaining a microbiological culture sample. These products were saved in -80oC mechanical freezers until reinfusion.

High dose chemotherapy: High dose Melphalan was given to five multiple myeloma patients, and BEAM (BCNU, VP-16, Ara-C, melphalan) was administered to 11 patients with lymphoma (Table 1).

Table 1: Clinical features of the patients			
Number of the patients, n			16
Age (median, range), years			39 (24-62)
Sex (male/female)			9 / 7
Diagnosis of the patients	Lymphoma		11
	Multiple myeloma		5
Disease status of the patients at the transplantation		Lymphoma	8
		Multiple myeloma	0
		Lymphoma	0
		Multiple myeloma	2
		Lymphoma	3
		Multiple myeloma	3
Mobilization regimens		Lymphoma	0
		Multiple myeloma	3
		Lymphoma	12
		Multiple myeloma	1
Conditioning regimen		Lymphoma	11
		Multiple myeloma	0
		Lymphoma	0
			5

Table 2: Features of peripheral blood stem cell products.	
Pre-freeze total CD34+ cell dose (x106/kg; median, range)	4.9 (2.51 - 9.8)
Volume of product (ml; median, range)	867.8 (740 - 2390)
Duration of storing at -800C* (day; median, range)	66.1 (67.5 - 204)
Duration of infusion (min; median, range)	48.1 (15 - 150)
*= In autologous transplanted patients	

Infusion of PBSCs and monitoring of the patients: At 24 - 48 hours after the end of the conditioning regimens, HSC products were infused in autologous transplanted patients.

Patient care: Starting on the 5th day, patients who had auto-PBSCT received daily dose of 5 µg/kg G-CSF until the neutrophil count was ≥ 3×109/L. Throughout the aplastic phase, all of the patients received antiviral and antifungal prophylaxis. Prophylactic treatment with levofloxacin was given to 14 patients. Patients who developed neutropenic fever were treated according to established guidelines. Neutrophil and platelet engraftments were defined as the first day in which the former exceeded 0.5x109/L and the latter exceeded 20x109/L (without the need for platelet transfusion) for three consecutive days.

Blood samples: Peripheral blood samples of the patients were drawn every day from the 6th day after stem cell infusion until the third day after the neutrophil engraftments were achieved. For this, 2 ml of blood was collected into EDTA-containing tubes (vacuette K2 EDTA) by the vacutainer method at the pre-determined times from patients in the supine position.

CD34+ HSC enumeration: CD34+ cell enumeration was performed by flow cytometry (BD FACS-

Calibur, BD Biosciences, San Jose, CA, USA) immediately after peripheral blood samples were taken. All samples were analyzed using BD Procount Progenitor Cell Enumeration kit (catalog no: 340498, BD Biosciences, San Jose, CA, USA). The kit consisted of one test and one absolute control counting tube (TruCOUNT, BD Biosciences, San Jose, CA, USA), each containing a certain number of lyophilized fluorescent beads. In addition, ready-to-use reagents were provided. Briefly, 50µl of sample was mixed with 20µl of CD45-FITC/CD34-PE. Tubes were incubated for 15 min in the dark. Next, 450 µl of FACS buffer was added to lyse red blood cells, and the tube was incubated for additional 30 minutes in the dark. After incubation, the samples were analyzed byflow cytometry. The results are reported as the absolute number of CD34+ cells.

Statistical analysis: The student T test was used for two-group comparisons of the numeric variables. The Pearson correlation coefficient was calculated for variables with a normal distribution. The correlation between the engraftment periods and other variables was studied using the Spearman's rank correlation coefficient. The Mann-Whitney U test, Kruskal-Wallis Variance Analysis and Friedman Variance Analysis were used for variables a without normal distribution. Values of $p<0.05$ were accepted to be statistically significant, and the results are given as median (range). The data were analyzed using computer software (SPSS 16.0, SPPS, Inc., Chicago, IL).

Results

The median CD34+ cell number of the HSC products was 4.9 (2.51 - 9.8) x 106/kg body weight. Products from autologous transplanted patients were stored for a median of 67.5 (18 - 204) days at - 80°C.

The median time to neutropenia was 3.5 (0 – 6) days. All but one patient experienced neutropenic fever. The febrile period could be treated empirically with antibiotics against gram negative and gram-positive microorganisms in 8 patients. Antibiotic modifications were considered due to the lack of response to the first empiric antibiotics in 11 patients. 12 patients were treated with an antifungal therapy during the febrile period. Neutrophil and thrombocyte engraftments were achieved at a median of 10.5 (9 - 16) and 11 (7 - 22) days, respectively.

In the auto-PBSCT group, CD34+ cell counts were lowest during the first 10 days (0.38, - 0.36, – 0.36, - 0.41, and 0.36 /µL). The CD34+ cell counts began to increase on the 11th day (0.45 / µL), and the highest value was achieved (0.87 /µL) on the 14th day after then they began to decrease. The values on the 16th day were lower than the values on the 6th day (0.38/µL vs. 0.26/µL (Figure 1). We documented evidence of mobilization in only one patient (6.25%). An inverse correlation was determined between CD34+ cell levels on the 9th day and neutrophil engraftment ($r=-0.63$) ($p=0.007$) (Figure 2). Compared with patients who had CD34+ cell counts ≥1.6/µL on the 9th day, the patients with CD34+ cell counts<1.6/µL had delayed neutrophil (9.5 vs. 11) ($p=0.01$) (Figure 3) engraftment.

The patients who took levofloxacin prophylaxis had significantly lower levels of CD34+ cells between days 8 and 12 compared to the patients who did not take it ($p<0.05$).

Discussion

As far as we know, there is no study in the literature which searches for the mobilization after auto-

PBSCT. In the present study, we documented very low levels of successful mobilization (6.2%) after auto-PBSCT. Based on this study, collecting purged in-vivo stem cell products (due to the conditioning regimen) following the first auto-PBSCT is not a logical and practical method for tandem transplantation.

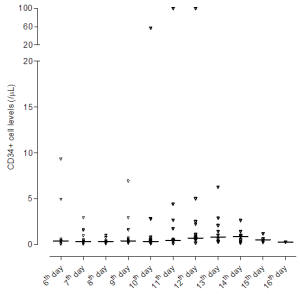

Figure 1:

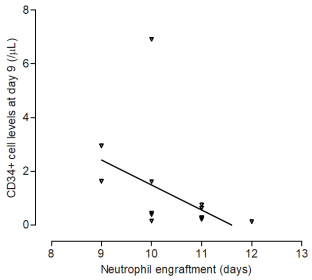

Figure 2:

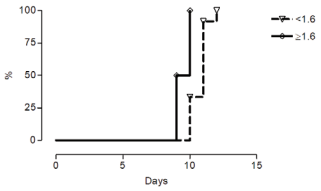

Figure 3:

In the literature, there is evidence that a prolonged high-dose treatment can reduce tumor burden and exert an in vivo purging effect on PBSC collections.[4,5] Stem cells collected after a sequence of chemotherapy contained significantly fewer plasma cells than those harvested upfront after a single high-dose drug administration. Different strategies were studied in MM patients for HSC harvesting and tandem transplantation.[6-8] In our study, we could not document a high rate of mobilization. An inverse correlation between CD34+ stem cell levels on the 9th day and neutrophil engraftment was found. This inverse correlation was quite an important finding as it indicated that higher CD34+ stem cell levels by the 9th day, resulted in earlier neutrophil engraftment. Additionally, four patients with CD34+ stem cell counts of ≥1.6 /µL on the 9th day had earlier neutrophil engraftment. Because of these two reasons, quantification of CD34+ stem cell levels on the 9th day can provide important information for auto-PBSCT. The evaluation of this finding in subsequent prospective studies may add valuable contributions to the literature. We also observed that peripheral CD34+ stem cells were decreased in the group treated with levofloxacin. This adds valuable information to the existing literature because we could not find any information about levofloxacin treatment. This study had a limitation of small number cases and a heterogeneous group. Large prospective cohort studies are required to determine this hypothesis.

Conclusion

In conclusion, although our study group was small and heterogeneous, the rate of successful stem cell mobilization was very low following auto-PBSCT. For this reason, it is not effective and practical to collect stem cells following the first transplantation.

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