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Research Article / Araştırma Makalesi

The Optimal Time of Peripheral Blood Hematopoietic Stem Cell Collection for Autologous Transplantation May Be Predicted By Immature Granulocytes and Nucleated Red Blood Cells

Otolog Nakil İçin Periferik Kan Hematopoietik Kök Hücre Toplamanın Optimal Zamanı, Olgunlaşmamış Granülositler ve Çekirdekli Kırmızı Kan Hücreleri Tarafından Tahmin Edilebilir

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Abstract

The time of initiating stem cell collection is very important for successful transplantation and is determined according to peripheral blood CD34+ cell count analysed by flow cytometry. In this study, we aimed to find the role of new complete blood count parameters to determine the optimal time of stem cell collection. Eighty-six patients who underwent stem cell mobilization were included in the study. Peripheral blood CD34+ cells and complete blood count were analysed on the same day. Patients with peripheral blood CD 34+ cell counts ≤ 20/μL and > 20/μL were determined as Group 1 and Group 2, respectively. The difference of CBC parameters between 2 groups and the relationship of these parameters with peripheral blood CD34+ cell counts were evaluated. It was found that immature granulocytes ratio, nucleated red blood cells ratio and count were positively correlated with peripheral blood CD34+ cell count and these parameters were significantly different between two groups (P< 0.001, P= 0.011 and P= 0.012, respectively). The new index formulated by using immature granulocytes and nucleated red blood cells ratios was significantly different between two groups (P<0.001), and had the highest diagnostic accuracy to determine the time of stem cell collection (AUC= 0.766). Immature granulocytes and nucleated red blood cells can be used to determine the time of peripheral blood stem cell collection faster and cheaper compared to flow cytometry.

Keywords: Autologous transplantation; Stem cell mobilization; CD34+ cells; Immature granulocytes; Nucleated red blood cells

Özet

Başarılı bir nakil için kök hücre toplamaya başlama zamanı çok önemlidir ve bu zaman akış sitometrisi ile analiz edilen periferik kan CD34+ hücre sayısına göre belirlenir. Bu çalışmada, kök hücre toplamanın optimal zamanını belirlemek için yeni tam kan sayımı parametrelerinin rolünü bulmayı amaçladık. Çalışmaya kök hücre mobilizasyonu uygulanan 86 hasta dahil edildi. Aynı gün periferik kan CD34+ hücreleri ve tam kan sayımı yapıldı. Periferik kan CD 34+ hücre sayısı ≤ 20/μL ve > 20/μL olan hastalar sırasıyla Grup 1 ve Grup 2 olarak belirlendi. İki grup arasındaki tam kan sayımı parametreleri farkı ve bu parametrelerin periferik kan CD34+ hücre sayısı ile ilişkisi değerlendirildi. İmmatür granülosit oranı, çekirdekli eritrosit oranı ve sayısının periferik kan CD34+ hücre sayısı ile pozitif korelasyon gösterdiği ve bu parametrelerin iki grup arasında anlamlı olarak farklı olduğu bulundu (sırasıyla P< 0.001, P= 0.011 ve P= 0.012). Olgunlaşmamış granülositler ve çekirdekli kırmızı kan hücreleri oranları kullanılarak formüle edilen yeni indeks, iki grup arasında önemli ölçüde farklıydı (P<0.001) ve kök hücre toplama zamanını belirlemek için en yüksek tanı doğruluğuna sahipti (AUC= 0.766). İmmatür granülositler ve çekirdekli kırmızı kan hücreleri, akış sitometrisine göre periferik kan kök hücre toplama zamanını daha hızlı ve daha ucuza belirlemek için kullanılabilir.
Anahtar Kelimeler: Otolog nakil; Kök hücre mobilizasyonu; CD34+ hücreleri; Olgunlaşmamış granülositler; Çekirdekli kırmızı kan hücreleri

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1. Introduction

Autologous peripheral blood stem cell (PBSC) transplantation is widely performed for the treatment of hematological malignancies (1). Hematopoietic stem cells are mostly collected from peripheral blood by apheresis. The optimal time for stem cell collection varies between patients and the time of initiating stem cell collection should be determined according to peripheral blood stem cell count (2). A minimum of $2x10^6$ $CD34^+$ cells/kg is needed for a successful transplant. The amount of CD34+ cells in peripheral blood should be at least 20/µL in order to collect this amount (3). The gold standard method to determine the amount of peripheral blood stem cells is flow cytometry. However, this method is costly and time consuming (4), and experienced personnel is necessary.

In Sysmex XN 1000 (Sysmex Corporation, Kobe, Japan), automatic complete blood count (CBC) analysers, immature granulocytes (IG) are counted in white blood cell differential (WDF) channel and nucleated red blood cells (NRBC) are counted in white cell nucleated (WNR) channel with fluorescent flow cytometry method. IG is expressed as the sum of metamyelocytes, myelocytes and promyelocytes, while NRBC reflects the increase in erythropoietic activity.

In this study, it was aimed to determine the roles of IG, NRBC, routine components of CBC parameters and peripheral blood CD34+ cell counts for the prediction of the optimal time of stem cell collection.

2. Materials and Methods

In this study, conducted with the approval of the Eskisehir Osmangazi University Noninterventional Clinical Research Ethical Committee (30.03.2021, no: 22). Local Ethics Committee according to the principles of the Declaration of Helsinki, 86 adult patients who underwent stem cell mobilization between October 2018 and August 2020 were included. Mobilization and stem cell collection records were evaluated retrospectively. Peripheral blood CD34+ cells were analysed with flow cytometer (Becton Dickinson Biosciences FACS Calibur, California, US) according to ISHAGE guidelines (5). CBC analyses were performed by Sysmex XN 1000 (Sysmex Corporation, Kobe, Japan) on the same day with flow cytometry which was also the first day of stem cell collection. Patients were divided into two groups according to the peripheral blood CD 34+ cell count (Group 1 ≤20/µL, Group 2 >20/µL). Values of haemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), count and distribution of leukocytes, counts of platelet, immature granulocytes (IG) and nucleated red blood cells (NRBC), IG ratio (IG count x 100 / leukocytes count) and NRBC ratio (NRBC count x 100 / erythrocyte count) were evaluated. The differences of these parameters between the groups and the relationships of significant parameters with peripheral blood CD34+ cell counts were examined. Additionally, the significance of the parameters was evaluated with logistic regression analysis and an index was created that may be used to determine the time of stem cell collection by using these significant parameters.

Statistical analysis

Normally and non-normally distributed continuous variables were expressed as mean ± standard deviation and median (25^{th}-75^{th} quartile), respectively. Student's t-test and Mann-Whitney U test were applied to the continuous variables as appropriate. Spearman correlation analysis was performed to show the relationship between the variables.

Variables that were significant in the univariate logistic regression analysis were used in the multivariate logistic regression analysis to create an index that could be used to determine the time of stem cell collection. Sensitivity and specificity were calculated for the zero cut-off value. Receiver Operating Characteristic (ROC) analysis was performed and the areas under the curve (AUC) were evaluated to determine the diagnostic accuracy of the CBC parameters and the created index. All data analyses were performed with SPSS package program. *P*

values less than 0.05 were accepted as statistically significant.

3. Results

The median age was 56 years and 48.8% were female. The diagnoses were multiple myeloma in 56, non-Hodgkin lymphoma in 18, Hodgkin lymphoma in 8, plasma cell leukaemia in 2, amyloidosis in 1 and multiple plasmositoma in 1 of the patients. Mobilization regimens were cyclophosphamide and granulocyte colony stimulating factor (G-CSF) in 55, DHAP (cisplatin, cytarabine, dexamethasone) and G-CSF in 7, ICE (iphosphamide, carboplatine, etoposide) and G-CSF in 12, hyper CVAD/methotrexate-cytarabine and G-CSF in 5, plerixafor and G-CSF in 2, G-CSF alone in 4 and etoposide and G-CSF in 1 of the patients.

The descriptives and CBC results of the groups are shown in Table 1. It was found that IG ratio, NRBC ratio and NRBC counts were statistically significantly different between the groups ($P< 0.001$, $P= 0.011$ and $P= 0.012$, respectively). IG ratio, NRBC ratio and NRBC counts were positively correlated with peripheral blood CD34+ cell counts (Table 2). The index was formulated as -1.7 + (IG% x 0.12) + (NRBC% x 2) by multivariate logistic regression analysis with the cut-off point of zero. This index was also statistically significantly different between the groups ($P <0.001$). According to these results, the parameter with the highest diagnostic accuracy was the new index with an AUC of 0.766 (0.665 – 0.867) (Table 3, Figure 1). The sensitivity of the index was 76% and specifity was 72% with a cut-off point of zero.

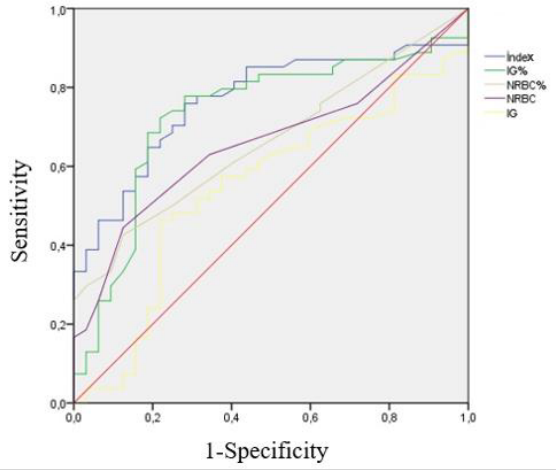

Figure 1. ROC curves of parameters

Table 1. Descriptive features and CBC results of the groups

	Group 1 (n=32)	**Group 2** (n=54)	***P***
Peripheral blood CD34+ cell count (10^6/L)	13 (10-15)	49 (29-101)	<0.001
Age (year)	58 (48-65)	56 (48-63)	0.734
Gender (F/M)	15/17	27/27	0.779

Haemoglobin (g/dL)	10 ± 1.2	10 ± 1.6	0.731
Hematocrit (%)	31 ± 4.2	31 ± 5.0	0.996
MCV (fL)	89 ± 7.1	88 ± 6.4	0.347
MCH (pg)	30 ± 2.9	30 ± 2.3	0.669
MCHC (%)	33 ± 1.4	34 ± 1.4	0.372
Leukocyte (10^9/L)	9.29 (4.55-12.71)	7.95 (3.81-14.11)	0.456
Neutrophil (10^9/L)	7.98 (3.34-10.59)	5.68 (2.40-11.2)	0.286
Lymphocyte (10^9/L)	0.47 (0.27-1.12)	0.53 (0.32-0.85)	0.943
Monocyte (10^9/L)	0.95 (0.53-1.46)	1.02 (0.68-1.77)	0.655
Eosinophil (10^9/L)	0.04 (0.02-0.11)	0.05 (0.02-0.12)	0.710
Basophil (10^9/L)	0.03 (0.01-0.06)	0.01 (0.02-0.04)	0.682
Platelet (10^9/L)	37 (26-78.5)	56 (30-87.75)	0.218
IG ratio (%)	8.4 (6.75-10.63)	12.75 (9.95-16.23)	**<0.001**
IG (10^9/L)	0.62 (0.49-1.26)	0.97 (0.4-1.7)	0.472
NRBC ratio (%)	0.1 (0-0.28)	0.25 (0.01-0.73)	**0.011**
NRBC (10^9/L)	0.01 (0-0.02)	0.02 (0.01-0.05)	**0.012**
Index	-0.39 (-0.66-0.09)	0.41(-0.04-1.35)	**<0.001**

Mann-Whitney U test or Student's t-test was used for between group comparisons. MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular hemoglobin concentration, IG: immature granulocytes, NRBC: nucleated red blood cells.

Table 2. Correlation between the parameters of complete blood count and peripheral blood CD 34^+ cell count

		IG%	**IG**	**NRBC%**	**NRBC**
Peripheral blood CD34+ cell count	r	0.418	0.245	0.383	0.438
	P	<0.001	0.023	<0.001	<0.001

Spearman correlation analysis was performed. IG: immature granulocytes, NRBC: nucleated red blood cells.

Table 3. ROC analysis

Parameter	**AUC (95% CI)**	***P***
Index	0.766 (0.665-0.867)	<0.001
IG%	0.732 (0.619-0.845)	<0.001
NRBC%	0.663 (0.550-0.775)	0.005
NRBC	0.660 (0.546-0.774)	0.006
IG	0.547 (0.419-0.674)	0.473

IG: immature granulocytes, NRBC: nucleated red blood cells.

4. Discussion and Conclusion

Studies on detecting the presence of stem cells in peripheral blood date back to the 1950s (6). It was shown that stem cells enter the circulation, and then repopulate in the bone marrow (7), and blood stem cells have begun to be used for the treatment of hematological malignancies. The success of hematopoietic stem cell transplantation depends on the infusion of an adequate dose of hematopoietic stem cell and successful repopulation of these cells in the bone marrow (8). Therefore, the time of initiating stem cell collection is very important for successful treatment.

The time of initiating stem cell collection should be determined according to peripheral CD34+ stem cell count (2), and the most widely used method used to estimate the amount of stem cells is the peripheral blood CD 34+ cell count determined by flow cytometry. Although flow cytometry is the gold standard method to determine the amount of peripheral CD34+ stem cell count, it is an expensive, time consuming method and requires technical expertise. Therefore, researchers have begun to search for different methods in determining the optimal stem cell collection time. A hematopoietic progenitor cell (HPC) count can be performed on Sysmex XN series automated blood count analyser, has become an alternative and successful method for the quantification of peripheral CD34+ stem cell (9-14). This measure has been shown to reduce costs (14), however, HPC measurement requires a WPC channel which means a new cost. That's why, in this study, we aimed to investigate the role of not stem cell but also young cells, IG and NRBC, in determining the time of stem cell collection.

NRBCs are immature red blood cell precursors and they are not found in the peripheral blood of healthy adults. Increased hematopoietic stress may cause to expel from the bone marrow into the circulation. NRBCs can be detected in healthy new-borns, rapid blood loss and hemolysis, hematologic diseases as chronic myeloid leukaemia, acute leukaemia, and myelodysplastic syndromes. Increased peripheral NRBC has been associated with high risk of death in different clinical status (15).

The IG fraction includes promyelocytes, myelocytes, and metamyelocytes and these cells are increased in inflammation, infection, trauma, necrosis, steroid use and pregnancy. IG are measured on many instruments and have been suggested as a marker for infection and sepsis (16).

NRBC count is performed by WNR channel with leukocytes and basophil counts, and IG count is by WDF channel with neutrophil, lymphocyte, monocytes and eosinophil counts by fluorescence flow cytometry. These advanced clinical parameters (17) are offered as part of CBC analysis in Sysmex.

In this study, it was found that IG ratio, NRBC ratio and NRBC counts are significantly positively correlated with peripheral blood CD34+ cell counts and IG ratio, NRBC ratio, NRBC counts and the new index are significantly different between 2 groups which formed according to peripheral blood CD 34+ cell count. This new index, created using IG% and NRBC%, has the highest diagnostic accuracy in the determination time of stem cell collection. Various biochemical pathways may be evaluated together by approaches that the use of multiple markers and higher accuracy is achieved than using the markers alone as in this study.

Although it is clear that there is a close relationship between peripheral blood CD34+ cell counts and IG and NRBC levels, the sensitivity and specificity were slightly lower than expected. It was thought that this situation might be related to the rise of these premature cells after stem cell development. A higher sensitivity and specificity can be obtained if the new studies are performed by comparing the change of IG and NRBC with the peripheral blood CD34+ cell counts.

To the best of our knowledge, this is the first study evaluating the use of IG, NRBC and derived index to determine the optimal time of peripheral blood stem cell collection. These results should be confirmed by the large number of patients in prospective studies, because CBC analysis is faster, more practical and cost-effective than flow cytometry analysis.

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Ethics

Ethics Committee Approval: The study was approved by Eskişehir Osmangazi University Noninterventional Clinical Research Ethical Committee (Decision no: 22, Date:30.03.2021).

Abstact of this study was accepted as 'publication only' at the European Hematology Association Congress in 2021 and the study was presented as an oral presentation at the 47th National Hematology Congress in Turkey in 2021.

Informed Consent: The authors declared that it was not considered necessary to get consent from the patients because the study was a retrospective data analysis.

Authorship Contributions: E.K. and E.G. designed the research study. E.K., E.G. and H.U.T. performed the research. H.K. and I.O.A. analysed the data. E.K. and E.G. wrote the paper. H.K., H.U.T. and I.O.A. edited the paper. All authors have read and approved the final manuscript.

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