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Comparison of stem cell CD45/34 fluorescence intensity with stem cell mobilization in patients under and over 65 years of age

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Abstract

In our research, we attempted to compare CD45/34 MFI (Mean Fluorescent Intensity) in patients younger and over 65 years of age with hematopoietic stem cell mobilization (HSCM).The research involved a group of 76 individuals who had different types of cancer and were identified at the Bone Marrow Transplantation (BMT) Unit of Anadolu Medical Center Hospital from 2015 to 2016. To mobilize HSCs, participants were administered daily granulocyte colony-stimulating factor (G-CSF) (10 µg/kg/day) under the skin for 7-8 days. Calculating the appropriate level of peripheral blood (PB) CD34+ took into account the patients' WBC (White Blood Cell) counts. Our research revealed that HSCM patients above 65 had statistically greater CD45/34 MFI values than those under that age. Although the age factor for HSCM is important, according to our findings, age is not seen as a negative mobilization factor for HSCM in patients aged 65 and over, and should be supported by larger studies. Our research revealed that patients over 65 who underwent HSCM had statistically greater CD45/34 MFI values than younger patients. Given the decline in SC production observed in individuals aged 65 and above, along with the potential for other accompanying diseases, our results hold promise for elderly patients. However, it is important to validate these findings through multicenter studies with a larger patient population, while also taking into account the presence of other subsequent diseases in the patients under observation.

Keywords: Stem cell mobilization, mean fluorescent intensity, CD45/34, hematopoietic stem cell transplantation

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Kök hücre CD45/34 floresan yoğunluğunun 65 yaş altı ve üstü hastalarda kök hücre mobilizasyonu ile karşılaştırılması

Özet

Araştırmamızda, hematopoietik kök hücre mobilizasyonu (HKHM) uygulanan 65 yaş altı ve üstü hastalarda CD45/34 MFI (Ortalama Floresan Yoğunluğu) değerlerini karşılaştırmayı amaçladık. 2015-2016 yılları arasında Anadolu Sağlık Merkezi Hastanesi Kemik İliği Transplantasyonu (KİT) Ünitesi'nde farklı kanser türlerine sahip 76 kişilik bir grupla çalıştık. HKH'leri harekete geçirmek için katılımcılara 7-8 gün boyunca cilt altından günlük granülosit koloni uyarıcı faktör (G-CSF) (10 µg/kg/gün) uygulandı. Uygun periferik kan (PK) CD34+ seviyesinin hesaplanmasında hastaların WBC (Beyaz Kan Hücresi) sayıları dikkate alınmıştır. Araştırmamız 65 yaş üstü HKHM hastalarının bu yaşın altındakilere kıyasla istatistiksel olarak daha yüksek CD45/34 MFI değerlerine sahip olduğunu ortaya koymuştur. HKHM için yaş faktörü önemli olmakla birlikte, bulgularımıza göre 65 yaş ve üzeri hastalarda yaş HKHM için olumsuz bir mobilizasyon faktörü olarak görülmemekte olup daha büyük çalışmalarla desteklenmelidir. Araştırmamız, HKHM uygulanan 65 yaş üstü hastaların genç hastalara kıyasla istatistiksel olarak daha yüksek CD45/34 MFI değerlerine sahip olduğunu ortaya koymuştur. KH üretiminde 65 yaş ve üzeri bireylerde gözlenen düşüş ve eşlik

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eden diğer hastalıkların potansiyeli göz önüne alındığında, sonuçlarımız yaşlı hastalar için umut vaat etmektedir. Bununla birlikte, bu bulguların çok merkezli çalışmalarla daha geniş bir hasta popülasyonuyla doğrulanması ve gözlem altındaki hastalarda diğer müteakip hastalıkların varlığının da dikkate alınması önemlidir.

Anahtar kelimeler: Kök hücre mobilizasyonu, ortalama floresan yoğunluğu, CD45/34, hematopoetik kök hücre transplantasyonu.

1. Introduction

Some hematological cancers can be cured with hematopoietic stem cell transplantation (HSCT). Peripheral SCs are mostly used as SC sources for autologous and allogeneic SC transplants. HSCM required for HSCT is provided by the G-CSF mobilization agent [1]. G-CSF blocks the CXCR4 receptor expressed by HSCs. HSCs are released into PB when the interaction of stromal-derived factor-1 (SDF-1) expressed by bone marrow (BM) stroma and CXCR4 receptor is inhibited [2]. The success of HSCM is determined by the number of CD34+ SCs that enter the peripheral blood. In our study, the MFI values of CD45/34+ cells were used. MFI is frequently used to measure the expression level of CD45/34 antigens across samples and cell types in flow cytometry. The CD45/CD34 antigen is a human leukocyte antigen belonging to the leucocyte common antigen (LCA) family, with a molecular weight ranging from 180 to 220 kilodaltons (kDa). It is expressed on all human leukocytes and is observed to have low levels of expression on hematopoietic progenitor cells. The CD45/CD34 marker is the main determinant of HSCs and is frequently used in the flow cytometer device in hematology laboratories to calculate the number of SCs

In one parameter histograms, data is expressed as either the % of a population's cells or the MFI. MFI value defined as the intensity of the fluorescent signals of the antigen-bound monoclonal test antibodies, is a surrogate marker for antigen density in the cell.

Several factors, including age, medical condition, chemotherapeutic regimen used for mobilization, previous sessions of chemotherapy or radiation treatment, and the time elapsed since the last chemotherapy cycle, can influence the process of HSCM [3]. Because various research has shown that elderly patients have lower mobilization capacity than younger patients, the results that are now available are inconsistent. In the study of Tempescul et al. (2010) with 359 patients, it was found that the HSCM success rates of patients below and above 65 years of age were similar (92% compared to 90.6%, respectively), However, a significant difference was observed in the number of CD34+ HSCs collected from the patients between the two age groups. Despite the significantly lower median number of CD34+ HSCs collected from the population aged 65 and over, this was still sufficient to perform one or more autologous SC transplants [4]. In light of all this information, we compared the CD45/34 MFI values in HSCM in individuals below and above the age of 65.

2. Materials and methods

From 2015 to 2016, a total of 76 patients were enrolled in a study conducted at the Bone Marrow Transplantation Unit of Anadolu Health Center Hospital. These patients had various diagnoses, with 37 (48.7%) cases of Multiple Myeloma (MM), 33 (43.4%) cases of Lymphoma, 2 (2.7%) cases of Testicular Cancer (Testicular CA), 3 (4%) cases of Solid Tumors, and 1 (1.3%) case of Acute Myeloid Leukemia (AML). All patients underwent HSCM procedures and were included in the study (Table 1).

Table 1. Diagnostic distributions

Diagnosis	Frequency	Percent
MM	37	48.68
Lymphoma	33	43.42
Testicular CA	2	2.63
Solid Tumors	1	1.32
AML	1	1.32
MM	2	2.63
Total	76	100

Multiple Myeloma (MM), Testicular Cancer (Testicular CA), Acute Myeloid Leukemia (AML)

The HSCM process usually begins with high-dose chemo and/or radiation therapy lasting about a week or two. This treatment aims to remove unwanted cells and make room for new cells to come in HSCT. Then, depending on the previous chemotherapy regimens received by the patients, mobilization chemotherapy is applied, which usually lasts for

1-2 days. One day after mobilization chemotherapy, G-CSF (10 µg/kg/day) is administered subcutaneously to patients daily for 7-8 days for HSCM. After 7-8 days, a PB sample was taken from the patient who came to the hospital to calculate the HSC and WBC count in the EDTA tube. The WBC count of the patients was considered to calculate the desired quantity of PB CD34+.

For CD45 FITC/34 PE staining with flow cytometer, 100 µl of the patient's mobilized PB sample was placed in the flow tube, and then 20 µl of CD45 FITC/CD34 PE (BD Bioscience, Cat No. 341071) antibody was added to it. Immediately after incubation in the dark for 20 min,2 mL of lysis solution was added to remove the erythrocytes in the sample. It was incubated for 10 min in the dark. After incubation, samples were washed twice with phosphate-buffered saline (PBS) for 5 minutes at 1800 rpm. Samples were resuspended with PBS and analyzed on a flow cytometer (BD Bioscience Facs Calibur).

The amount of CD34+ cells stained in the flow cytometer was multiplied by the number of total WBCs of the patient and the number of CD34 positive cells required for adequate mobilization is 10 per mL. In addition, the CD45/34 MFI given by the flow cytometer device was recorded in the analyzes performed for SC PB CD34 + on the day of HSCM. When the desired CD34+ SC count is obtained, the patient's SC is collected in the apheresis unit. The CD45/34 MFI number, which directly affects the number of SCs, was taken from the flow cytometer device, not the number of SCs calculated in the study. Flow cytometry analysis was performed on the first day of mobilization. The number of CD45/34 MFIs is associated with the number of SCs, and a high one indicates high mobilization (Figure 1)

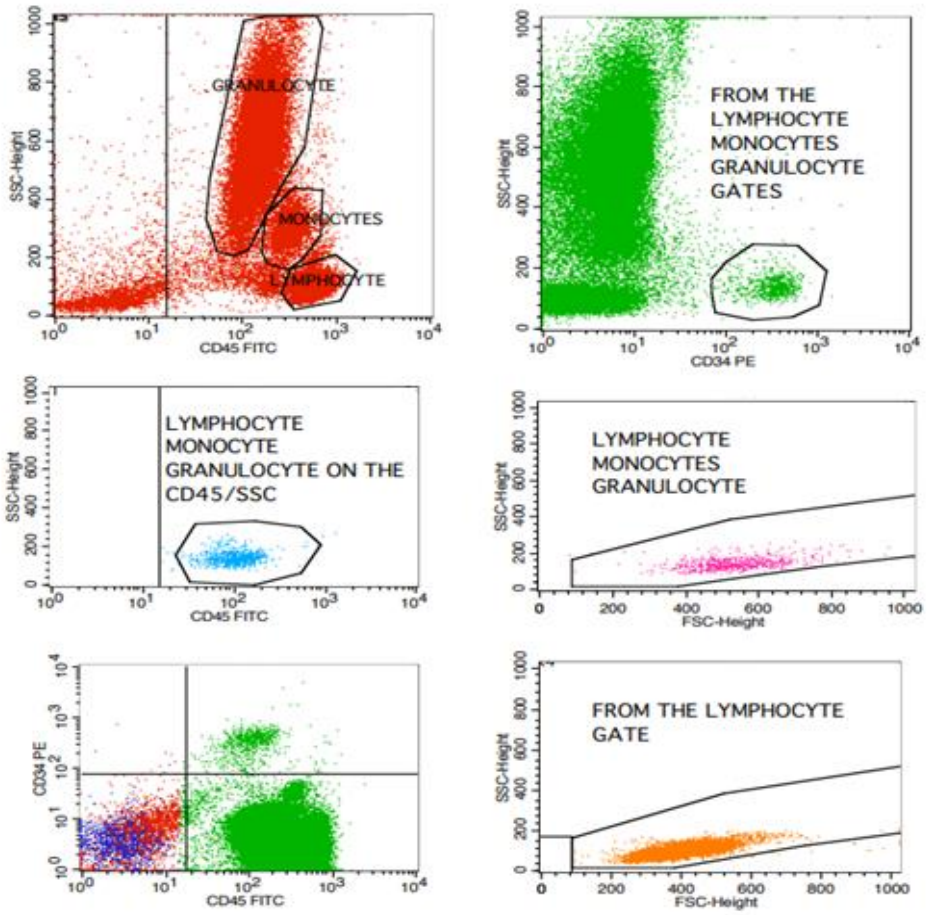

Figure 1. The representative flow cytometry plots (with gating strategy). The 2nd plot (green) gives the CD34 MFI percentage, and the 3rd plot (blue) the CD45 MFI percentage

Briefly, in our study, a technique called flow cytometry was used to calculate CD45/34 MFI (Mean Fluorescent Intensity) values. This process is specifically used to measure the intensity of antigens (such as CD45 and CD34) on the surface of cells. The process starts as described above by first preparing cell samples and staining them with special antibodies. The stained cells are then placed in a flow cytometer and exposed to a laser beam. The fluorescent light emitted by the antibodies was measured by the instrument and recorded as MFI values. These values reflect antigen levels on the surface of the cells, providing information on various biological and clinical conditions. For detailed methodology and flow cytometry techniques, the book [5] "Flow Cytometry: Principles and Applications (2007)" can be consulted. This book comprehensively covers the basic techniques used in flow cytometry, including

sample preparation, staining, flow cytometry analysis and data interpretation. It also provides detailed information on how MFI values are calculated.

The frequency and percentage were used to summarize the quantitative data, while the median (also represents the second quartile (Q_2), first quartile (Q_1), third quartile (Q_3), minimum (Min), and maximum (Max)) were used to summarize the quantitative variables. Independent group assessments were made using the Mann-Whitney U test and effect sizes were reported using rank-biserial correlation coefficients. JASP (Version 0.16.3) statistical software was used for statistical analysis. Statistical inferences were made at 5% significance level.

3. Results

Patients with HSCM were 44 (57.9%) men and 32 (42.1%) women (Table 4). While 52 of the patients were under the age of 65, 24 of the patients included in the study were over the age of 65. (Table 3). The 76 individuals who were a part of the research had an average age of 54.97. (17-77) (Table 2). There is a statistically significant difference between age groups in terms of CD45 and CD34 (respectively p=0.043 and 0.014). CD45 MFI distribution in the >=65 age group (Median=364.5, Q_1=279.25, and Q_3=394.25) is statistically significantly higher than in the <65 age group (Median=295.5, Q_1=221.5, and Q_3=361). CD34 MFI distribution in the >=65 age group (Median=424, Q_1=350.75, and Q_3=610.25) is statistically significantly higher than in the <65 age group (Median=342.5, Q_1=283, and Q_3=431.25). However, the effect sizes are low (respectively r_{rb}=-0.292 and -0.354) (Table 3). The distribution chart of CD45 MFI and CD34 MFI by age group is shown in Figure 2.

Table 2. Descriptive statistics for age

	n	Mean	SD	Min	Q_1	Median (Q_2)	Q_3	Max
Yaş	76	54.97	14.65	17	48.75	58.00	66.00	77

SD: Standard Deviation, Q: Quartile

Table 3. Comparison of age groups in terms of CD45 MFI and CD34 MFI

	<65 age (n=52) Median (Q_1-Q_3) [Min-Max]	>=65 age (n=24) Median (Q_1-Q_3) [Min-Max]	W*	p	Rank-Biserial Correlation**
CD45	295.5 (221.5 - 361) [137 - 766]	364.5 (279.25 - 394.25) [156 - 798]	442.0	0.043	-0.292
CD45	342.5 (283 - 431.25) [104 - 1038]	424 (350.75 - 610.25) [237 - 1313]	403.0	0.014	-0.354

*Mann-Whitney U test statistics. Q: Quartile

**For the Mann-Whitney test, effect size is given by the rank biserial correlation.

Table 4. Comparison of gender groups in terms of CD45 MFI and CD34 MFI

	Male (n=44) Median (Q1-Q3) [Min-Max]	Female (n=32) Median (Q1-Q3) [Min-Max]	W	p	Rank-Biserial Correlation**
CD45	335.5 (276.75 - 407.25) [151 - 798]	278 (205 - 367.25) [137 - 623]	898.0	0.042	0.276
CD34	363.5 (299.75 - 503.25) [203 - 1038]	359 (282.25 - 458.75) [104 - 1313]	789.5	0.371	0.121

*Mann-Whitney U test statistics. Q: Quartile

**For the Mann-Whitney test, effect size is given by the rank biserial correlation.

The statistical analysis shows that there is a statistically significant difference between gender groups in terms of CD45 MFI but there is no statistical significance in terms of CD34 MFI (respectively p=0.042 and 0.371). CD45 MFI distribution in the male group (Median=335.5, Q_1=276.75, and Q_3=407.25) is statistically significantly higher than in the female group (Median=278, Q_1=205 and Q_3=367.25). However, the effect size is low (r_{rb}=0.276) (Table 4). The distribution chart of CD45 MFI and CD34 MFI by gender groups is shown in Figure 3.

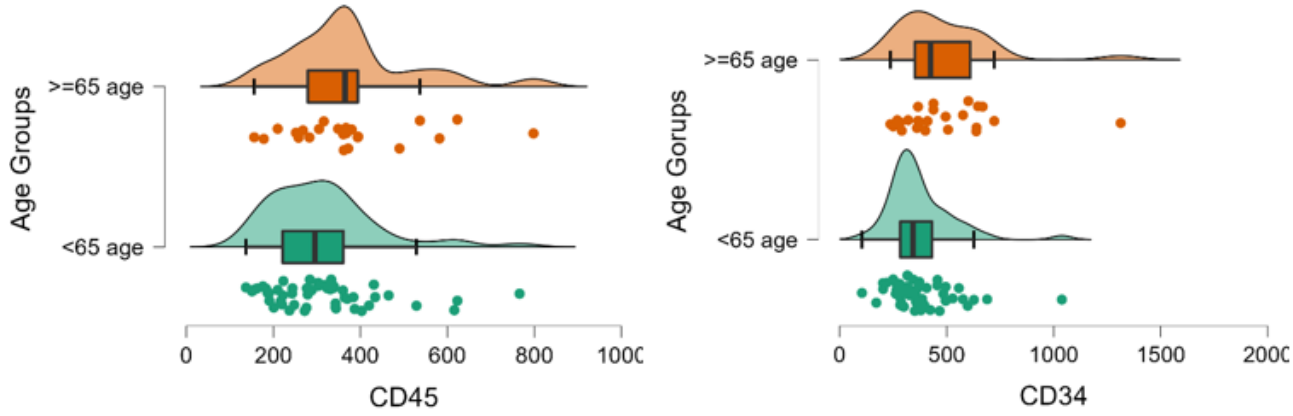

Figure 2. Distributions of CD45 MFI and CD34 MFI in the age groups

Note: Points represent the observed (measured) values. Box-plot graphs represent the minimum, maximum, and first, second, and third quartiles. And one-sided violin graphs represent the estimated density of the distribution of the interested variable.

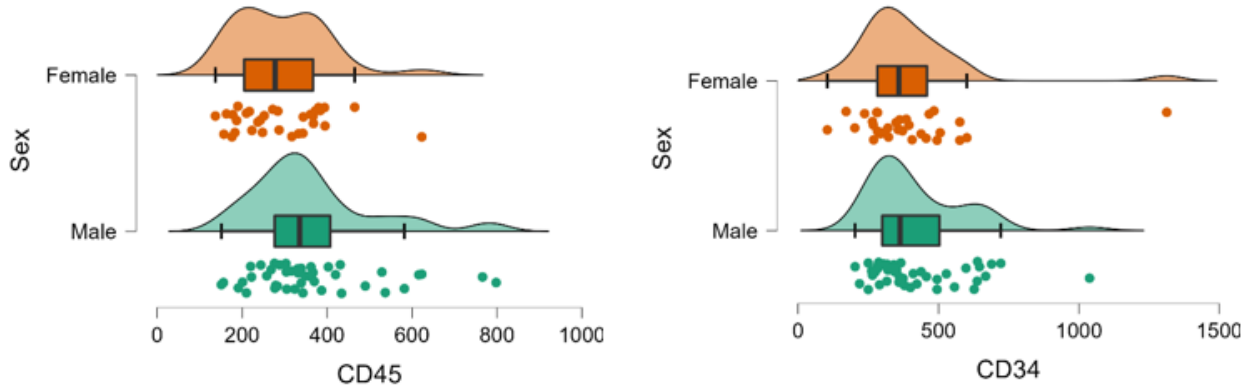

Figure 3. Distributions of CD45 MFI and CD34 MFI in the gender groups

Note: Points represent the observed (measured) values. Blot graphs represent the minimum, maximum, and first, second, and third quartiles. And one-sided violin graphs represent the estimated density of the distribution of the interested variable.

4. Conclusions and discussion

Patients with HSCM were 44 (57.9%) men and 32 (42.1%) women (Table 4). While 52 of the patients were under the age of 65, 24 of the patients included in the study were over the age of 65. (Table 3). The 76 individuals who were a part of the research had an average age of 54.97. (17-77) (Table 2). There is a statistically significant difference between age groups in terms of CD45 and CD34 (respectively p=0.043 and 0.014). CD45 MFI distribution in the >=65 age group (Median=364.5, Q_1=279.25, and Q_3=394.25) is statistically significantly higher than in the <65 age group (Median=295.5, Q_1=221.5, and Q_3=361). CD34 MFI distribution in the >=65 age group (Median=424, Q_1=350.75, and Q_3=610.25) is statistically significantly higher than in the <65 age group (Median=342.5, Q_1=283, and Q_3=431.25). However, the effect sizes are low (respectively r_{rb}=-0.292 and -0.354) (Table 3). The distribution chart of CD45 MFI and CD34 MFI by age group is shown in Figure 2.

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It is known that age-related phenotypic changes in cells are particularly important in elderly individuals. Such changes can affect cell function and response to therapies, making them crucial in studies involving elderly populations.

Age-related changes in cell biology and their impact on disease and treatment are discussed in "Brocklehurst's Textbook of Geriatric Medicine and Gerontology" [6] and how aging affects cellular function and response to therapies. Also according to Gabali, A. [7], it was explained that age-related phenotypic changes in haematolymphoid cells may be particularly important in the diagnosis of disease. According to the findings in this literature, it highlights the importance of detailed cellular analysis and the potential impact of age on cellular characteristics, which may affect the efficacy of treatments and diagnostic accuracy in elderly patients. This underlines the need for age-specific considerations in medical research, particularly studies focusing on haematopoietic stem cell mobilisation and related therapies.

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