EXPLORATION FOR POSSIBLE USE OF GM-CSF RECEPTOR NUMBERS ON MYELOID CELL POPULATIONS AS A PREDICTIVE VALUE FOR STEM CELL TRANSPLANTATIONS

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ABSTRACT

EXPLORATION FOR POSSIBLE USE OF GM-CSF RECEPTOR NUMBERS ON MYELOID CELL POPULATIONS AS A PREDICTIVE VALUE FOR STEM CELL TRANSPLANTATIONS

Peripheral hematopoietic stem cell transplantation is a method used in the treatment of many diseases, especially hematological malignancies. With the method called apheresis, the patient's own stem cells (autologous) or stem cells from a matched healthy donor (allogeneic) are collected and transplantation is performed under appropriate conditions. Evaluation of the quality of the received apheresis product is very important in terms of both measuring the collection efficiency and monitoring the transplantation dynamics. Although the gold standard $CD34^+$ stem cell count is accepted today, the centers' evaluation and calculation criteria differ between centers. This study aimed to determine the number and expression of GM-CSFR (CD116) and to measure its usability as an additional predictive parameter to CD34 in the evaluation of the quality of the apheresis product. The percentage of CD116, Mean Fluorescent Intensity (MFI) and GM-CSF receptor number were determined in apheresis products of autologous and allogeneic donors and in peripheral blood of healthy donors who did not receive Granulocyte Colony-Stimulating Factor (G-CSF). Obtained data were compared both between myeloid cells and between study groups. While the percentage of CD116 did not show a significant difference both on myeloid cells and between groups, MFI of CD116 and GM-CSF receptor numbers were detected on monocytes (mean MFI value 4,8, mean GM-CSFR number 9.560 ABC/Cell) with the highest concentration in myeloid cells, and in apheresis products of allogeneic donors among the groups (mean MFI value 5,0, mean GM-CSFR number 9.965 ABC/Cell). There was no significant correlation between CD34 and CD116 in apheresis products, but the highest MFI of CD116 (mean 3,79) and GM-CSF receptor number (mean 7.506 ABC/Cell) were observed in apheresis products from allogeneic donors with the highest leukocyte (mean 204.513/µL) and platelet (mean 165 x 10^3/µL) counts. In autologous donors, a positive correlation was found between the percentage of CD116 and the number of WBCs in the apheresis product ($p = 0,008$). In conclusion, CD116 can be used as a predictive factor in evaluating the quality of apheresis products by providing information about myeloid cell content.

ÖZET

KÖK HÜCRE TRANSPLANTASYONLARINDA MYELOİD HÜCRELER ÜZERİNDE BULUNAN GM-CSF RESEPTÖR SAYILARININ PREDİKTİF DEĞERİNİN ARAŞTIRILMASI

Periferik hematopoetik kök hücre nakli, başta hematolojik maligniteler olmak üzere birçok hastalığın tedavisinde kullanılan bir metoddur. Aferez adı verilen yöntemle hastanın kendisine ait kök hücreler (otolog) veya uygun sağlıklı bir donöre ait kök hücreler (allojenik) toplanarak uygun koşullar altında transplantasyon işlemi gerçekleştirilir. Alınan aferez ürününün kalitesinin değerlendirilmesi hem toplama verimliliğinin ölçülmesi hem de transplantasyon dinamiklerinin takibi açısından oldukça önemlidir. Günümüzde altın standart $CD34^+$ kök hücre sayısı kabul edilse de merkezler arası değerlendirme ve hesaplama kriterleri farklılık gösterir. Bu çalışmanın amacı, GM-CSFR (CD116) sayısı ve ifadesinin tespit edilerek, aferez ürününün kalitesinin değerlendirilmesine CD34'e ek prediktif bir parametre olarak kullanılabilirliğini ölçmekti. Otolog ve allojenik donörlere ait aferez ürünlerinde ve Granülosit Koloni-Stimülan Faktör (G-CSF) almamış sağlıklı donörlere ait periferik kan örneklerinde CD116 yüzdesi, Ortalama Floresan Yoğunluğu (MFI) ve GM-CSF reseptör sayısı belirlendi. Elde edilen veriler hem myeloid hücreler arasında hem de çalışma grupları arasında karşılaştırıldı. CD116 yüzdesi hem myeloid hücreler üzerinde, hem de gruplar arasında anlamlı bir fark göstermezken CD116 MFI'ı ve GM-CSF reseptör sayısı myeloid hücrelerde en yoğun monositlerde (ortalama MFI değeri 4,8, ortalama GM-CSFR sayısı 9.560 ABC/Hücre), gruplar arasında ise en yoğun allojenik donörlere ait aferez ürünlerinde tespit edildi (ortalama MFI değeri 5,0, ortalama GM-CSFR sayısı 9.965 ABC/Hücre). Aferez ürünlerinde CD34 ile CD116 arasında anlamlı bir korelasyon saptanmadı ($p = 0,6$) ancak en yüksek CD116 MFI'ı (ortalama 3,79) ve GM-CSF reseptör sayısı (ortalama 7.506 ABC/Hücre) en yüksek lökosit (ortalama 204.513/µL) ve platelet (ortalama 165 x 10^3/µL) sayısına sahip olan allojenik donörlerden alınan aferez ürünlerinde gözlemlendi. Otolog donörlerde, CD116 yüzdesi ile aferez ürünündeki WBC sayısı arasında pozitif bir korelasyon saptandı ($p = 0,008$). Sonuç olarak, CD116, myeloid hücre içeriği hakkında bilgi vererek aferez ürünlerinin kalitesinin değerlendirilmesinde prediktif bir faktör olarak kullanılabilir.

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LIST OF SYMBOLS/ABBREVIATIONS

ABC	Antigen binding capacity
ALL	Acute lymphoid leukemia
AML	Acute myeloid leukemia
B-ALL	B-cell lymphoblastic leukemia
CD	Cluster of differentiation
CD2	T-cell surface antigen cd2 precursor
CD3	T-cell surface glycoprotein cd3 precursor
CD7	T-cell surface antigen cd7 precursor
CD10	Common acute lymphocytic leukemia antigen
CD19	B-lymphocyte antigen cd19 precursor
CD34	Hematopoietic progenitor cell antigen cd34
CD38	Cyclic ADP ribose hydrolase
CD45	Lymphocyte common antigen
CD114	Granulocyte colony-stimulating factor receptor
CD116	Granulocyte-macrophage colony-stimulating factor receptor
CD138	Syndecan-1
CEBPA	CCAAT/enhancer-binding protein alpha
CEC	Collection efficiency coefficient
CFU-GM	Granulocyte-macrophage colony-forming unit
CO_2	Carbon dioxide
COVID-19	Coronavirus disease of 2019
CXCL12	C-X-C motif chemokine ligand 12
CXCR4	C-X-C chemokine receptor type 4
EPO	Erythropoietin
FLT3	Fms like tyrosine kinase 3
FS	Forward scatter
G-CSF	Granulocyte colony-stimulating factor
G-CSFR	Granulocyte colony-stimulating factor receptor
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GM-CSFR	Granulocyte-macrophage colony-stimulating factor receptor
GVHD	Graft-versus-host disease

GVT	Graft-versus-tumor
HLA	Human leukocyte antigen
HLA-DR	HLA class II histocompatibility antigen gamma chain
HIV	Human immunodeficiency virus
HSC	Hematopoietic stem cell
HSCT	Hematopoietic stem cell transplantation
IL-1	Interleukin-1
IL-3	Interleukin-3
IL-6	Interleukin-6
IL-11	Interleukin-11
ITD	Internal-tandem duplications
MFI	Mean fluorescence intensity
MNC	Mononuclear cell
MGDF	Megakaryocyte growth and development factor
MMP9	Metalloproteinase 9
MPN	Myeloproliferative neoplasm
NPM1	Nucleophosmin 1
NK	Natural killer
O_2	Oxygen
q-PCR	Quantitative polymerase chain reaction
RT-PCR	Real-time polymerase chain reaction
SS	Side scatter
STAT	Signal transducer and activator of transcription
T-ALL	T-cell lymphoblastic leukemia
TNF-α	Tumour necrosis factor alpha
TPO	Thrombopoietin
TP53	Tumor protein P53
WHO	World Health Organization

1. INTRODUCTION

1.1. HEMATOPOIETIC SYSTEM AND ITS DISORDERS

1.1.1. Hematopoiesis

Stem cells can renew themselves without dividing and give rise to various cell types. Stem cells are examined in two main categories as embryonic and adult stem cells. Embryonic stem cells originate from the inner cell mass of a blastocyst and are classified as totipotent, pluripotent, multipotent and unipotent according to their differentiation stages. In the third week of embryonic development also called gastrulation, the cells in the bilaminar disc form three germ layers, endoderm, mesoderm and ectoderm. Endoderm cells differentiate into lung, liver pancreas, stomach and intestine cells, mesoderm cells differentiate into bone, bone marrow blood and muscle cells, ectoderm cells differentiate into nerve and skin cells. Hematopoietic stem cells (HSCs) that reside in bone marrow, originate from the mesodermal layer can self-renew and differentiate into all hematopoietic cells. HSCs differentiate according to the characteristics of the microenvironment they are in (Figure 1.1.). Growth factors, transcription factors, cytokines, hormones and extracellular signals determine the fate of the cell [1]. The first step of hematopoiesis begins with the differentiation of pluripotent stem cells into multipotent progenitor cells. Self-renewing multipotent hematopoietic stem cells differentiate into $CD34^+$ multipotent hematopoietic progenitor cells. These progenitor cells generate common lymphoid precursor and common myeloid precursor cells. The differentiation process continues with the differentiation of multipotent cells into precursor cells, mainly myeloblasts, erythroblasts, megakaryoblasts. Fully differentiated mature blood cells enter the peripheral circulation [2]. Erythrocytes are responsible for the transport of O_2 and CO_2 while leukocytes are the most important component of a body's defense mechanism. Leukocytes can be examined in two groups: granulocytes and agranulocytes. Granulocytes contain neutrophils, eosinophils, and basophils which kill pathogens during an infection. Monocytes and lymphocytes have no granules in their cytoplasm. Similar to granulocytes, monocytes help defend the body against infectious diseases also can differentiate into dendritic cells. Lymphocytes can be classified as antibody-producing B lymphocytes and virus-infected cell killer T lymphocytes. B

lymphocytes mature in bone marrow while T lymphocytes mature in the thymus. Natural killer cells also called large granular lymphocytes, kill virus-infected cells by releasing perforin and granzyme. Platelets derived from megakaryocytes repair damaged blood vessels and help blood clotting [3].

Hematopoietic growth factors are widely used in neutropenia, anemia treatment and stem cell mobilization. Cytokine named Granulocyte Colony-Stimulating Factor (G-CSF) is released by monocytes, macrophages, fibroblasts and endothelial cells, binds G-CSF Receptor (G-CSFR) and stimulates the production of neutrophils while Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) is released by macrophages, T lymphocytes, mast cells, natural killer cells, fibroblasts and endothelial cells, binds GM-CSF Receptor (GM-CSFR) and stimulates the production of neutrophils, monocytes, and eosinophils [4,5]. Erythropoietin (EPO) used in the treatment of anemia is secreted from the kidney and liver in response to hypoxic stress and stimulates the production of erythrocytes in the bone marrow [6]. IL-1, IL-3, IL-6, IL-11, thrombopoietin (TPO) and megakaryocyte growth and development factor (MGDF) are cytokines with thrombopoietic effects by inducing the production of megakaryocytes and platelets. TPO and IL-11 are widely used in the treatment of chemotherapy-related thrombocytopenia [5].

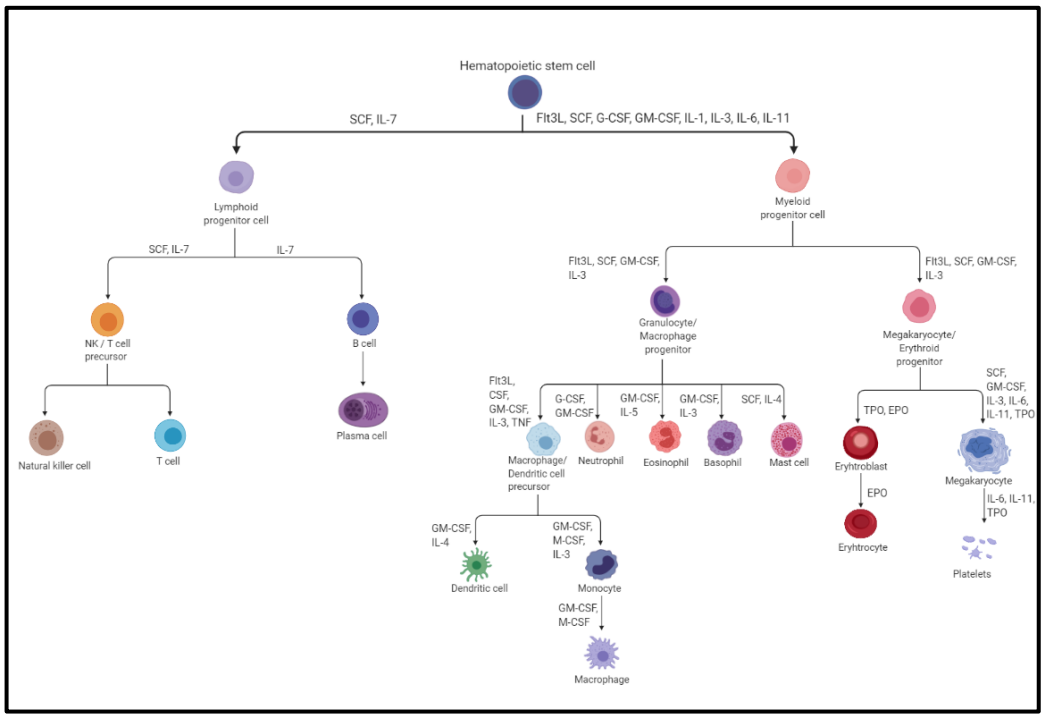


Figure 1.1. Hematopoiesis scheme [3]

1.1.2. Hematological Malignities

A genetic or/and epigenetic error and uncontrolled proliferation of cells during the differentiation of stem cells into mature blood cells cause the formation of hematological cancers such as leukemia, lymphoma and multiple myeloma. Chromosomal translocations, activation of oncogenes, inhibition of tumor suppressor genes and disruption of apoptosis-related genes are the main factors in the formation of hematological malignancies [7]. Leukemias are classified according to the type of cell they originate from (myeloid or lymphoid) and their genotypic and phenotypic characteristics. According to the World Health Organization (WHO) classification updated in 2016, leukemias are defined as Myeloproliferative Neoplasms (MPN), Myelodysplastic/Myeloproliferative Neoplasms (MDS/MPN), Acute Myeloid Leukemia (AML) and related neoplasms, Blastic plasmacytoid dendritic neoplasm, B-cell Lymphoblastic leukemia (B-ALL) and T-cell Lymphoblastic Leukemia (T-ALL) [8]. In Multiple myeloma, antibody-producing plasma cells increase in an uncontrolled manner, preventing the production of other blood cells. Abnormal plasma cells produced in the bone marrow secrete a uniform type of immunoglobulin protein called “paraprotein”. It is characterized by the waist, back and bone pain, lytic lesions in the bones and increased abnormal plasma cells in the bone marrow. While the risk of developing the disease increases with age, it is usually diagnosed over 60 years of age [9]. Lymphoma is caused by B and T lymphocyte abnormalities and is characterized by enlarged lymph nodes. It is divided into two main categories: Hodgkin Lymphoma and Non-Hodgkin Lymphoma. HIV, Epstein-Barr virus infection and autoimmune diseases are risk factors for lymphoma [10]. In a global study, between 2006 and 2016, there was a 26 percent increase in leukemia cases and a 45 percent increase in Non-Hodgkin Lymphoma cases. While leukemia was identified as the 9th deadliest cancer type, Non-Hodgkin Lymphoma was identified as the 10th deadliest cancer type [11]. As seen in Figure 1.2., according to a study comparing lymphoid and myeloid malignancies in Europe in 2010: the highest incidence of lymphoid malignancies was plasma cell neoplasms and the highest incidence of myeloid malignancies was acute myeloid leukemia [12] .

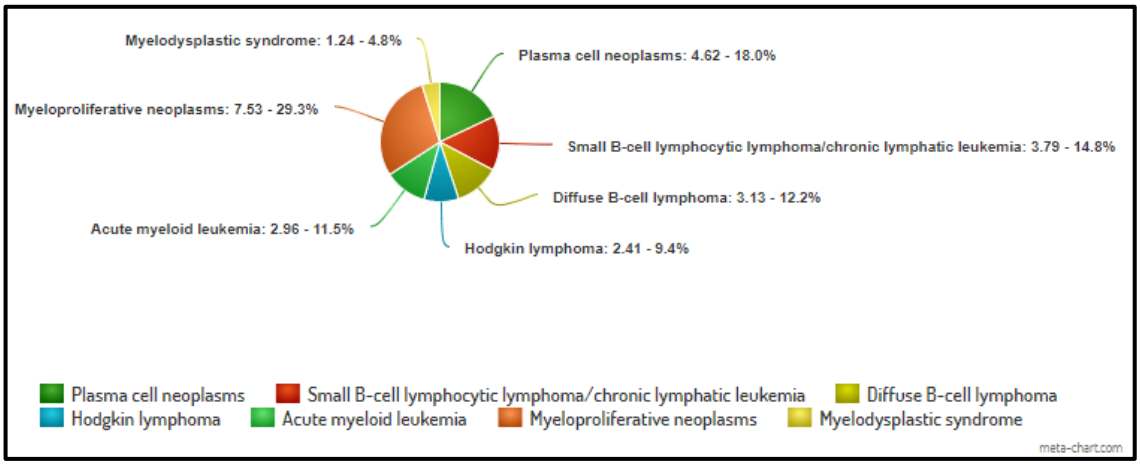


Figure 1.2. Incidence of hematologic cancers in Europe in 2010 [12]

1.1.3. Diagnosis and Treatment of Hemalotogical Malignities

Most hematological malignancies are caused by one or more damaged protein production as a result of a mutation in DNA or chromosomal rearrangements. At the first stage in the diagnosis of hematological cancers, biochemistry tests, especially total blood count, peripheral blood smear, molecular, cytogenetic and pathological tests are applied. Flow cytometry is the most widely used method for determining the subtype of the disease and evaluating the response rate to treatment. Acute myeloid leukemia is defined by the increase of myeloid blasts in peripheral blood and/or bone marrow. In cytogenetic tests, t (15; 17), t (8; 21), inv 16 or t (6; 16) positivity are important criteria in the diagnosis of AML [13]. According to the WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues revised in 2017, the blast percentage in peripheral blood or bone marrow should be ≥ 20 percent [14]. Mutations occurring in NPM1, FLT3/ ITD, TP53 and CEBPA genes are prognostic factors evaluated in determining favorable, moderate and poor-risk patient groups and direct treatment. Chemotherapeutic combinations such as cytarabine and anthracycline are administered to patients in the favorable risk group, allogeneic stem cell therapy is recommended for patients in the moderate and poor-risk groups. Acute lymphoblastic leukemia is characterized by increased immature lymphocytes in the bone marrow and the positivity of CD34, CD10 and CD19 markers on these cells is diagnostic for B-ALL, while CD2, CD3, CD7 and CD34 positivity are seen in T-ALL cases. In cytogenetic evaluations, the presence of t (9; 22) (Philadelphia chromosome) and t (12; 21) (TEL-AML1 fusion) aids

diagnosis and treatment. Allogeneic stem cell transplantation is recommended for relapse and chemotherapy-resistant patients [15]. In patients with multiple myeloma, the presence of t (4; 14), t (14; 16), t (14; 20), + 1q or del1p is considered to be a high risk in cytogenetic examinations. A high ratio of kappa/lambda light chain and high serum paraprotein levels indicate a poor prognosis. Plasma cells with $CD45^{low}CD138^{+}CD38^{+}$ expression detected in flow cytometry tests are important criteria in diagnosis. Nowadays, autologous stem cell-assisted high-dose chemotherapy treatment is a widely used method. Criteria such as the age of the patient, rate of response to treatment and cardiac function determine autologous stem cell applicability. Although autologous stem cell transplantation is generally recommended under the age of 65, it has been observed that it is also effective over the age of 80 with supportive treatments [16]. Lymph node biopsy and bone marrow biopsy are the main methods used in the diagnosis of lymphoma, which is highly prevalent in young adults between the ages of 20-40. A high proportion of $CD19^{+}$ B cells in the bone marrow and an increased kappa/lambda light chain ratio aid diagnosis. While chemotherapy and radiotherapy combinations are used in the treatment of lymphoma, autologous stem cell transplantation is applied to patients who are resistant to treatment or relapse [17].

1.2. STEM CELL TRANSPLANTATION AND MOBILIZATION

Stem cell transplantation is used in the treatment of mainly hematological diseases, genetic diseases, immunodeficiency diseases, solid tumor cancers and autoimmune diseases. Despite the main source of stem cells being considered to be bone marrow, nowadays stem cells are collected from the peripheral circulation with a process called apheresis. In the mid-1950, total body irradiation followed by administration of bone marrow cells from homologous animals was found to reduce the leukemia cells and restore hematopoiesis in a mouse leukemia model [18]. Stem cell transplantation is performed in three different types, syngeneic, autologous and allogeneic. The hematopoietic stem cell transplantation (HSCT) type may vary depending on the disease condition or severity (Table 1.1.).

Table 1.1. Diseases in which HSCT is used

Disease	Disease Condition	HSCT Type	Reference
Acute myeloid leukemia	After relapse In the first remission if there is a high risk cytogenetics Unresponsive to the first indication	Allogeneic Allogeneic Allogeneic	[14,18]
Acute lymphoblastic leukemia	After relapse In the first remission if there is high risk cytogenetics Unresponsive to the first indication	Allogeneic Allogeneic Allogeneic	[15,20]
Myelodysplastic Syndrome	Extremely high number of blasts or high risk cytogenetic or multiline cytopenias	Allogeneic	[21]
Chronic Myeloid Leukemia	Imatinib failure Accelerated phase Blast crisis	Allogeneic Allogeneic Allogeneic	[22,23]
Chronic Lymphocytic Leukemia	After relapse with a more aggressive phenotype or with high risk cytogenetics	Allogeneic	[24]
Aplastic Anemia	Critical Category	Allogeneic	[25]
Myeloproliferative Disorder	"Spent" phase with bone marrow failure or high-risk features with impending bone marrow failure or progression to acute leukemia	Allogeneic	[26]
Hemoglobinopathy	Disease state with high risk prognostic features	Allogeneic	[27]
Intermediate- and High-Grade Non-Hodgkin's Lymphoma	After relapse High-risk features in first remission	Autologous Autologous	[17,28]
Low-Grade Non-Hodgkin's Lymphoma	After first remission or relapse with aggressive behavior or Richter transformation	Autologous or Allogeneic	[28,29]
Hodgkin's Lymphoma	After relapse or no response to initial treatment	Autologous	[17,28]
Multiple Myeloma	In the first remission After relapse	Autologous	[16,30]
Neuroblastoma	First remission in advanced-stage	Autologous	[31]
Pediatric Central Nervous System Tumors	Aggressive malignancy	Autologous	[31,32]

1.2.3. Stem Cell Transplantation

There are three types of immunological HSCT based on donor: autologous, allogeneic and syngeneic. In autologous HSCT, auto means "self" and the patient is transplanted with self-collected cells. In allogeneic HSCT, cells collected from a donor with Human Leukocyte Antigen (HLA) -matched are transplanted to the patient. Syngeneic HSCT is a special condition where the donor and recipient are identical twins [33]. The diseases treated with transplant types and the advantages and disadvantages of each type are summarized in Table 1.2.

Table 1.2. Application areas, the advantages and disadvantages of different HSCT types [34]

HSCT Type	Application Areas	Advantages	Disadvantages
Allogeneic	Regulation of defective hematopoiesis and/or immunodeficiency, Establishing adoptive immunotherapy for cancer Facilitating high-dose chemotherapy	Provides GVT* effect Does not require the patient to have sufficient bone marrow Healthy stem cells Less cytotoxic agents	Lack of suitable donor GVHD** Risk of rejection
Autologous	Facilitating high-dose chemotherapy	No donor needed No GVHD	The tumor may contaminate the graft Previously damaged stem cells may be difficult to collect in sufficient numbers or may contribute to the risk of myelodysplasia
Syngeneic	Facilitating high-dose chemotherapy	No GVHD	No GVT

*GVT: Graft-Versus-Tumor, **GVHD: Graft-Versus-Host Disease

The transplant process can be divided into three basic steps: conditioning regimen, stem cell infusion, and supportive care. The purpose of the conditioning regimen is to kill cancer cells in autologous HSCT and suppress host immunity and kill cancer cells in allogeneic HSCT. Autologous stem cell infusion is performed to regulate myelosuppression and hematopoiesis. The main purpose of allogeneic stem cell infusion is to correct the immune deficiency or metabolic disorder due to abnormal hematopoietic cells in congenital metabolic diseases and immunodeficiencies. Supportive care in allogeneic HSCT is applied for the prevention and/or treatment of graft rejection with immunosuppressives, prevention and/or treatment of Graft-Versus-Host Disease (GVHD) with immunosuppressives, prevention and/or treatment of infections with antibiotics, compensation of anemia and thrombocytopenia with transfusions [35,36].

Allogeneic HSCT is a therapeutic option for patients with acute and chronic leukemia, myeloid dysplastic syndromes or aplastic anemia. In patients with acute myeloid and lymphoid leukemia, allogeneic HSCT is considered if leukemia does not respond to initial induction therapy or relapses after the initial response [35]. To ensure safe donor engraftment in allogeneic HSCT, it is necessary to select the HLA-matched donor. The q arm of the sixth chromosome contains the HLA gene complex. This complex consists of Class I (HLA-A, HLA-B and HLA-C) and Class II (HLA-DR, HLA-DQ and HLA-DP) genes [37]. The minimum condition for the highest survival rate is high-resolution DNA matching in HLA-A, HLA-B, HLA-C, and HLA-DRB1 (8/8 HLA match). Allele level mismatch is associated with worse transplant outcomes [38,39]. The fact that continuous control cannot be achieved with chemotherapy in patients in the first remission is accepted as an indication for HSCT. Since continuous control of chronic myeloid leukemia can be achieved with chemotherapy, currently HSCT is only considered in patients who are unresponsive to chemotherapy or who are in blast crisis. In patients with myelodysplastic syndrome, HSCT can be applied if there is an increased blast count, cytogenetic abnormality or cytopenia [35]. In allogeneic HSCT, high levels of T lymphocytes in the graft attack the skin, liver and gastrointestinal system and cause GVHD. TNF-α and IL-1 mediated dendritic cell activation in the host causes epithelial damage. Activation of cytotoxic T lymphocytes and natural killer (NK) cells in later stages, along with cytokine storm, damages tissues. Damage to the skin, gastrointestinal system mucosa and bile ducts occurring within 100 days after transplantation can become fatal if not intervened [40].

Autologous HSCT is a therapeutic option for patients with multiple myeloma, Hodgkin lymphoma or non-Hodgkin lymphoma in combination with high-dose chemotherapy. Autologous HSCT has been shown to provide a survival advantage for patients with multiple myeloma and is therefore administered after the first chemotherapy to reduce the disease burden. Autologous HSCT in lymphoma patients is scheduled after relapse. Autologous HSCT can be applied in diseases such as advanced stage neuroblastoma, recurrent or aggressive Hodgkin lymphoma, recurrent low-grade non-Hodgkin lymphoma and pediatric central nervous system tumors [35,41]. Although autologous HSCT is generally recommended under the age of 65, it can be applied over the age of 65. Studies have shown that in patients with multiple myeloma, reducing the dose of melphalan and performing tandem transplants can improve survival over the age of 65 [42]. The main goal of autologous HSCT is to compensate for the damage to the bone marrow after high-dose chemotherapy by giving the patient's cells. It is known that high-dose chemotherapy in addition to G-SCF increases peripheral stem cells during stem cell mobilization. Currently, the most commonly used chemotherapeutic agent in autologous HSCT is Cyclophosphamide (2–4 g/m^2) [43].

1.2.4. Stem Cell Mobilization

Peripheral blood HSCs are used as the main graft source in most patients who undergo autologous or allogeneic HSCT for the treatment of hematological malignancies. CD34 antigen is expressed as surface glycoprotein on HSCs and is a molecule used in clinical identification, number determination, and separation of stem cell precursors. It is known that the number of transplanted $CD34^+$ cells is correlated with engraftment kinetics [44]. CD34 antigen is found in 1-3 percent of bone marrow mononuclear cells (MNCs), while 0,01-0,05 percent of MNCs circulating in peripheral blood are CD34 positive [45]. Administration of recombinant human granulocyte colony-stimulating factor [G-CSF; Filgrastim (Neupogen, Amgen Inc, Thousand Oaks, CA)] to healthy donors at a dose of 10 µg/kg/day for 4-6 days can increase the HSC content of the bone marrow 1,5-2 fold [46,47]. In autologous HSCT, chemotherapeutic agents that cause transient aplasia such as high-dose Cyclophosphamide stimulate the migration of HSCs into peripheral blood during hematopoietic recovery [48]. Recombinant human G-CSF is the most commonly used cytokine for HSCT mobilization in patients treated with chemotherapy. By increasing the concentration of matrix

metalloproteinase 9 (MMP-9), G-CSF induces the release of proteolytic enzymes secreted from myeloid cells that break down the cytoadhesive connections in the bone marrow microenvironment [49]. G-CSF, when administered alone, ensures that the concentration of HSC in the blood reaches its maximum level approximately 5 days after administration. Recombinant human granulocyte-macrophage colony-stimulating factor [GM-CSF; Sargramostim (Leukine, Bayer Healthcare Pharmaceuticals, Wayne, NJ)] stimulates the proliferation and differentiation of hematopoietic precursor cells, monocytes and dendritic cells and induces their mobilization [50]. Combinations of G-CSF and GM-CSF are used as rescue regimes if mobilization with G-CSF alone is not successful [51]. Plerixafor (Mozobil, Genzyme Corporation, Cambridge, MA) is a selective and reversible C-X-C Chemokine Receptor Type 4 (CXCR4) antagonist. Plerixafor disrupts the interaction of CXCR4 with C-X-C Motif Chemokine Ligand 12 (CXCL12), allowing HSCs to be released into circulation. It is known that plerixafor enriches $CD34^+$ cell mobilization with G-CSF, and there is an average of 4,4-fold increase in $CD34^+$ cell mobilization compared to the use of G-CSF alone. Nowadays, plerixafor is recommended as a salvage therapy for patients who have failed the first peripheral blood mobilization trial [52].

1.3. APHERESIS: A TOOL FOR STEM CELL COLLECTION

Apheresis, which means removal/separation in Latin, is defined as the continuous processing of the blood taken from the person and separating it into its components. This separation process allows the desired ingredient to be retained and the remainder delivered to the person [53]. It was first used by John Jacob Abel in 1914 to separate the plasma portion of blood to treat toxemia in dogs with nephrectomy [54]. Apheresis is classified as donor apheresis, therapeutic apheresis and immunotherapy, according to the type of disease and its application (Figure 1.3.) [55]. The apheresis process takes between 1-4 hours and the cell collection process is performed through the vascular access. Nowadays, apheresis procedures are carried out with computerized, automatic devices in parallel with the developing technology, with sterile disposable sets, each of which is specially produced for the device to be used. With the help of pumps in the device during the procedure; the whole blood of the donor is taken, mixed with an appropriate anticoagulant at the specified ratio, anticoagulated whole blood is taken into the centrifuge system, the components in the system are separated into layers according to their specific gravity. The components are layered as

plasma at the top, then platelets, mononuclear cells, granulocytes and erythrocytes at the bottom. Device detectors detect the layers, the component whose separation is programmed is taken with some plasma and deposited in a separate bag. The other components of the blood are combined and returned to the donor or the patient [56,57]. In therapeutic apheresis, cell collection is performed by peripheral vascular access or a jugular catheter [55].

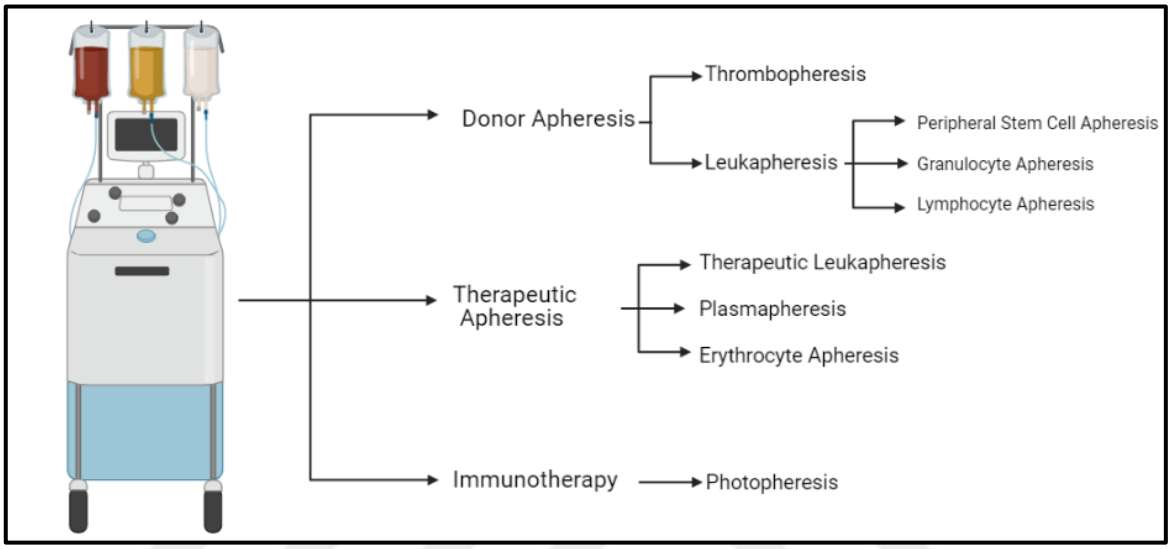


Figure 1.3. Types of apheresis [58]

1.3.1. Peripheral Stem Cell Collection

Mobilizing agents such as G-CSF and GM-CSF reduce the binding of $CD34^+$ cells in the bone marrow to adhesion molecules and to matrix metalloproteinases that change the pattern of the tissue matrix. These applications increase the number of $CD34^+$ cells in peripheral blood [50,52]. Patients who have received chemotherapy or healthy donors are given the appropriate dose of Granulocyte Colony-Stimulating Factor (G-CSF) (Filgrastim [Neupogen]) for 5 days to increase stem cell production in the bone marrow and enable them to enter the circulation. On the 5th day, $CD34^+$ stem cell count in peripheral blood is used to predict hematopoietic stem cell numbers in peripheral circulation [59]. Stem cell collection starts when the number of leukocytes in peripheral blood reaches $> 1 \times 10^3/\mu l$ and the number of $CD34^+$ cells reaches $> 20/\mu L$ [60]. With G-CSF given following chemotherapy, it was found that the amount of $CD34^+$ cells increased and neutrophil engraftment occurred

faster in the patient [61]. The apheresis process takes between 1-4 hours and the cell collection process is performed through the vascular access.

Nowadays, apheresis procedures are carried out with computerized, automatic devices in parallel with the developing technology, with sterile disposable sets, each of which is specially produced for the device to be used [62]. With the help of pumps in the device during the procedure; The whole blood of the donor is taken, mixed with an appropriate anticoagulant at the specified ratio, anticoagulated whole blood is taken into the centrifuge system, the components in the system are separated into layers according to their specific gravity. The components are layered as plasma at the top, then platelets, mononuclear cells, granulocytes and erythrocytes at the bottom [63,64]. Device detectors determine the layers, the desired component is taken and deposited in a separate bag. The other components of the blood are combined and returned to the donor or the patient [65].

1.3.2. Evaluation and Quantification of Collected Stem Cells

The CD34 antigen is found on the surface of primitive hematopoietic stem cells [66]. By tracking the $CD34^+$ cell count of apheresis products collected after mobilization, optimization and fast engraftment of the graft are provided [59]. It is known that there is a correlation between the number of $CD34^+$ infused and engraftment [67]. For successful engraftment, 2×10^6 $CD34^+$ cells/kg were accepted as the conservative dose required for rapid neutrophil and platelet healing, and the optimum amount of Granulocyte-Macrophage Colony-Forming Unit (CFU-GM) required for rapid hematopoietic recovery was found to be $20\text{-}50 \times 10^4$ CFU-GM/kg [48]. CFU tests are based on the ability of HSCs to colonize in a methylcellulose medium with special growth factors added. However, the test takes two weeks to complete, and the classification of the colonies formed requires careful attention [68]. Therefore, $CD34^+$ cell numbers are considered as the gold standard in the evaluation of apheresis products.

1.4. MEASUREMENT OF CD116, THE GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR RECEPTOR

GM-CSFR (Granulocyte-Macrophage Colony-Stimulating Factor Receptor) belongs to the hematopoietin/cytokine receptor superfamily and has a heteromeric structure. GM-CSFR consists of α (CDw116 [GM-CSFRα]) and β (GM-CSFRβ) subunits. While the alpha chain carries the binding site for GM-CSF, the beta chain is involved in signal transduction. The beta chain also acts as a receptor for IL-3 and IL-5. GM-CSFR has two different isoforms, membrane and secreted. As seen in Figure 1.4. the binding of GM-CSF to GM-CSFR increases cell production by inducing JAK2 (Janus Kinase-2) autophosphorylation. Autophosphorilated JAK2 activates STAT5/MAPK (Signal Transducer And Activator Of Transcription Protein-5/Mitogen-Activated Protein Kinase) thereby JAK/STAT/MAPK pathway and induces production and differentiation of granulocytes, monocytes and macrophages. The binding of GM-CSF to GM-CSFR induces dendritic cell maturation and activates STAT1 and STAT3 in neutrophils and STAT5 in monocytes. Activated STATs bind DNA and control the production, differentiation and function of monocytes, macrophages and neutrophils. Receptor expression is enriched in neutrophils, eosinophils, myeloid dendritic cells, plasmacytoid dendritic cells, classical monocytes, intermediate monocytes and non-classical monocytes [69,70]. Calculations based on Antigen Binding Capacity (ABC) show that the number of receptors on monocytes is four times higher than the number of receptors on neutrophils. In the peripheral blood of healthy donors, the number of GM-CSF receptors found in neutrophils was 3,23 ABC/Cell x 10^3 and 15,7 ABC/Cell x 10^3 in monocytes [71]. When the monocyte subgroups were examined, it was stated that the classical monocytes were found in the blood at an average of 80 to 90 percent, the intermediate monocytes at the rate of 2 to 5 percent, and the non-classical monocytes at the rate of 2 to 10 percent [72]. The expression of CD116 in monocyte subsets in the blood was examined and the highest expression of CD116 (approximately 100 percent) was observed in classical monocytes, followed by intermediate monocytes (90 to 100 percent). In non-classic monocytes, CD116 was expressed at a rate of approximately 65 to 85 percent. The highest Mean Fluorescence Intensity (MFI) of CD116 was detected in classic monocytes, followed by intermediate monocytes and the lowest in non-classic monocytes [73].

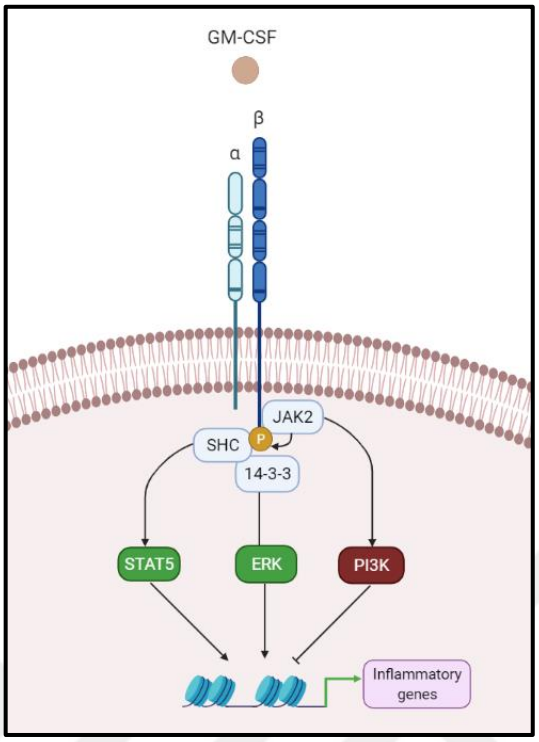


Figure 1.4. GM-CSF interacts with GM-CSFR and alters the expression of inflammatory genes [74]

Currently, there are limited studies about the CD116 levels in HSCs collected by apheresis and the use of CD116 to predict the quality of apheresis products. Therefore, we determined CD116 quantitative levels in apheresis samples from autologous and allogeneic donors and in peripheral blood from healthy donors and compared them between groups and myeloid cells. We evaluated its usability as a predictive value by investigating the correlation of CD116 levels with other parameters.

1.5. AIM OF THE STUDY

Evaluation of the amount and quality of stem cells in apheresis samples is vital for autologous and allogeneic transplantations. Nowadays, the $CD34^+$ number is the only parameter used in evaluating the quality of the apheresis product. In previous studies, the CFU-GM number was used along with the $CD34^+$ stem cell number. However, CFU-GM experiments are not widely used today because they require cell culture conditions and take approximately 14 days to get results therefore not suitable for routine work. Therefore, examining some other hematopoietic markers besides CD34 will provide new predictive factors for stem cell transplantation.

The main aim of this study is to determine the number of CD116 receptors on myeloid cells in apheresis samples. In addition, it was aimed to compare the factors such as the amount of G-CSF given to the patients and donors, leukocyte number, the number of $CD34^+$ cells, the chemotherapy received by the patient, age and gender with the number of CD116 receptors. Finally, it was aimed to explore the predictive value of CD116 in addition to CD34 in successful stem cell transplantations.

2. MATERIALS AND METHODS

2.1. MATERIALS AND CHEMICALS

All stages of this research are carried out in the Stem Cell Laboratory, Hematology Department, Yeditepe University Hospitals, Istanbul under the supervision and consultancy of Prof. Dr. Gülderen Yanıkkaya Demirel, the Head of Immunology Department. Apheresis samples were obtained from the Apheresis Center, Hematology Department, under the supervision and consultancy of Prof. Dr. Hasan Atilla Özkan.

2.1.1. Instruments

Therapeutic Apheresis Machine (Spectra Optia® - Terumo BCT, USA),

Navios EX Flow Cytometry (Beckman Coulter, USA),

Hematology analyzer (Sysmex XS-500i, Japan),

Centrifuge (Hettich Rotina 38R, Germany),

Vortex mixer (Ika V4D S000 Vortex, Germany),

Transport tank (Chart MVE, USA),

Controlled rate freezer (Planer KRYO 560-16, UK),

Liquid nitrogen tank (Cryotherm Apollo 50, Germany),

+4˚C refrigerator (Antech MP1-110, China),

+4˚C/-20˚C refrigerator (Siemens, Germany),

-80˚C freezer (Sanyo, Japan).

2.1.2. Equipments

12x75 mm flow tubes (Beckman Coulter, USA),

Micro pipettes 1000 µl (Thermo Scientific, USA), 200 µl (Thermo Scientific, USA), 20 µl (Gilson, USA), 10 µl (Gilson, USA).

2.1.3. Solutions and Kits

QIFIKIT Beads (Agilent Dako, USA, #K0078),

Stem-Kit Reagents; CD45, CD34, 7-AAD, StemCount Beads (Beckman Coulter, USA, #IM3630),

CD116 - PE Conjugated Antibody (Beckman Coulter, USA, #IM1977),

CD45 – A750 Conjugated Antibody (Beckman Coulter, USA, #A71119),

CD34 – PC7 Conjugated Antibody (Beckman Coulter, USA, #A51077),

CD3 - FITC Conjugated Antibody (Beckman Coulter, USA, #A07746),

CD19 - ECD Conjugated Antibody (Beckman Coulter, USA, #A07770),

VersaLyse Lysing Solution (Beckman Coulter, USA, #A09777),

IsoFlow Sheath Fluid (Beckman Coulter, USA, #8546859).

2.2. METHODS

All of the methods applied in this study were in accordance with standard operation procedures (SOPs) of Yeditepe University Koşuyolu İhtisas Hospital Stem Cell Laboratory.

A schematic representation of methods is presented below figure (Figure 2.1.):

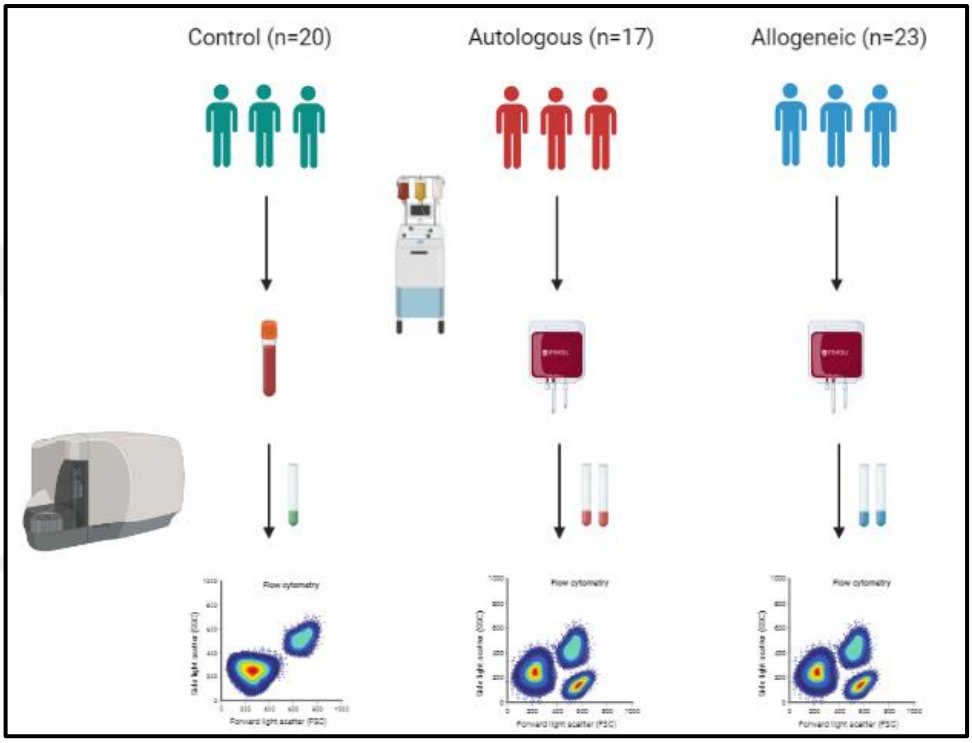


Figure 2.1. Schematic presentation of the workflow of collected peripheral blood and apheresis samples

2.2.1. Sample Collection

In this study, samples were obtained from Apheresis Unit, Yeditepe University Koşuyolu İhtisas Hospital.

The study titled "Determination of its Value as a Predictive Factor for Autologous Transplantation by Comparison of G-CSF Receptor and GM-CSF Receptor Numbers on Different Cell Groups in Peripheral Stem Cell Samples Collected by Apheresis" was approved by Yeditepe University Clinical Research Ethics Committee on 04.03.2020 (Decision no: 1172).

Stem cell mobilization for allogeneic donors was performed with G-CSF [Filgrastim (Neupogen, Amgen Inc, Thousand Oaks, CA)] at a dose of 10 µg/kg/day. Stem cell mobilization for autologous donors was performed with G-CSF [Filgrastim (Neupogen, Amgen Inc, Thousand Oaks, CA)] at a dose of 5 µg/kg/day in addition to chemotherapy in Lymphoma and Multiple Myeloma patients. Stem cell mobilization was performed with Plerixafor (Mozobil, Genzyme Corporation, Cambridge, MA) at a dose of 20 mg/mL in two Lymphoma and two Multiple Myeloma patients whose mobilization with G-CSF was not successful.

Peripheral stem cell collection was performed by the apheresis unit with a therapeutic apheresis machine (Spectra Optia® - Terumo BCT, USA). All apheresis samples were delivered to the laboratory in additive-free tubes. For comparison, two mL of peripheral blood samples from healthy volunteers who did not receive G-CSF were taken into an EDTA tube by the Biochemistry Laboratory and delivered to the laboratory.

2.2.2. Analysis of Apheresis Samples

Total blood counts of apheresis samples were determined on the Sysmex XS-500i Hematology Analyzer. All antibodies used in flow cytometric analysis are summarized in Table 2.1. Samples were separated into two tubes and labeled as “CD45/Isotype Control” to prevent autofluorescence and “CD45/CD34 Dual Positive” to detect absolute stem cell numbers. Ten µL of 7-AAD (Beckman Coulter, USA) and CD45/Isotype Control (Beckman Coulter, USA) were added to the control tube, 10 µL of 7-AAD and 15 µL of CD45/CD34 (Beckman Coulter, USA), added to detection tube and then mixed with 100 µL apheresis sample. Tubes were incubated for 15 minutes at room temperature. After incubation, erythrocyte lysis was performed with 1 mL of ammonium chloride containing VersaLyse Lysing Solution (Beckman Coulter, USA, #A09777). Cells were incubated for 10 minutes at room temperature. StemCount beads (Beckman Coulter, USA) were added to each tube and reading was performed on Navios EX Flow Cytometry (Beckman Coulter, USA). Viability, $CD45^{+}CD34^{+}$ absolute number and percentage in apheresis samples were analyzed with KALUZA Software Analysis. As shown in Figure 2.2., all gating adjustments and $CD34^{+}$ cell enumeration were performed in accordance with the International Society of Hematotherapy and Graft Engineering (ISHAGE) protocol [75]. According to the ISHAGE

protocol, all viable leukocyte cells labeled with FITC-conjugated CD45 antibody were analyzed in the FL-1 channel and the gate was labeled as "$CD45^+$" (Figure 2.2.a). HSCs labeled with PE-conjugated CD34 antibody in $CD45^+$ cells were analyzed in the FL-2 channel (Figure 2.2.b). To examine the $CD34^+$ cell cluster form, $CD45^{dim}$ cells in $CD34^+$ cells were examined in the FL-1 channel (Figure 2.2.c). The fluorescence and light scatter distribution of the $CD34^+$ cell cluster were shown in the Side Scatter (SS) and Forward Scatter (FS) channels within the $CD45^{dim}$ cells (Figure 2.2.d). Next, $CD45^+CD34^+$ cells were displayed for all events in channels FL-1 and FL-2 and StemCount beads were excluded (Figure 2.2.e). To prove the accuracy of the SS and FS voltage/gain parameters, the smallest viable lymphocyte cell population was examined in the SS and FS channels (Figure 2.2.f). By examining the projection of StemCount beads against time, it was shown that fluorescent beads passed through the flow cell homogeneously over time in singlet form (Figure 2.2.g). StemCount beads can be used for automatic calculation of $CD34^+$ absolute cell count, but the manual calculation was performed in this study. For detection of viable cells, 7-AAD positive cells were examined in the FL-3 channel and excluded (Figure 2.2.h). 7-AAD negative cells were gated and $CD45^+$ cells were analyzed through this gate.

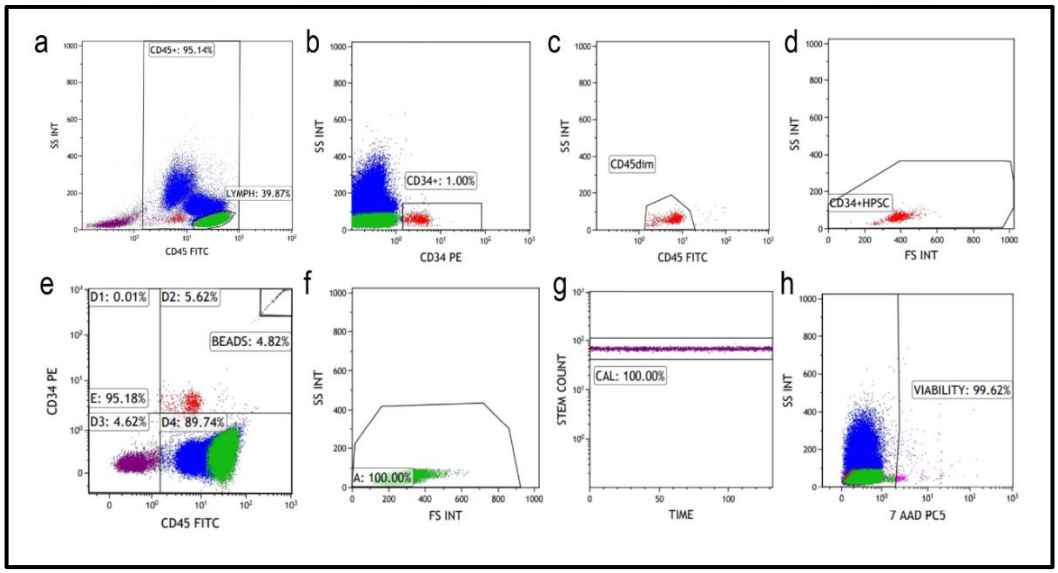


Figure 2.2. An apheresis sample stained with StemKit and analyzed on Navios EX Flow Cytometry. (a) Density plot displays viable $CD45^+$ cells, (b) Density plot displays viable $CD34^+$ HSCs, (c) Density plot displays $CD45^{dim}$ cells in $CD34^+$ HSCs, (d) Density plot displays SS and FS of $CD34^+$ HSCs in $CD45^{dim}$ cells, (e) $CD34^+CD45^+$ cells in all events, (f) SS and FS of lymphocytes, (g) StemCount beads versus time, (h) Viability of all cells

$CD45^+CD34^+$ absolute cell number calculated according to the formula below [75]:

$$CD34+\ Absolute\ Cell\ Count=\frac{WBC\ Count\times CD45+\ Cell\ Percentage\times CD34+\ Cell\ Percentage}{10.000} \quad (2.1)$$

Measuring mobilization success and determining collection efficiency are important factors in evaluating the quality of the apheresis product [76]. Therefore, the Collection Efficiency Coefficient (CEC) was calculated according to the formula below [76]:

$$\mathrm{CEC}(\%)=\frac{(CD34+cells\ /Kg\ of\ the\ recipient\ body\ weight)\times Recipient\ body\ weight(kg)}{Total\ volume\ processed\ (L)\times Peripheral\ blood\ CD34+\ cells\ /\ \mu L\times 10} \quad (2.2)$$

CEC was calculated and compared separately for apheresis samples collected from autologous and allogeneic donors.

Estimating how many $CD34^+$ cells can be collected in a daily apheresis procedure provides a great advantage for the donor and the apheresis unit. In a cohort of 307 allogeneic donors, Almeida-Neto et al. reported that the number of collected $CD34^+$ cells showed a linear correlation with the predicted $CD34^+$ cell yield [76]. The predicted $CD34^+$ cell yield was calculated using the CEC values calculated in the formula below [76]:

$$Number\ of\ CD34+\ cell\ yield\ (cells\ \mathrm{x}\ 10^6\ /kg)\ \mathrm{x}\ \left[\frac{(Peripheral\ blood\ CD34+\ cells\ /\ \mu L\ )\times CEC(\%)}{Recipient\ body\ weight(kg)}\right]\ \mathrm{x}\ Total\ volume\ processed\ (L) \quad (2.3)$$

The predicted $CD34^+$ cell was compared with the collected $CD34^+$ cell.

It is known that the main cause of GVHD in allogeneic transplantations is the host $CD3^+$ T lymphocytes [77]. In this study, the percentage of $CD3^+$ T lymphocytes and $CD19^+$ B lymphocytes in apheresis samples taken from allogeneic donors were determined and its correlation with CD116 was examined. For determination of B and T lymphocyte content, 100 µL of samples were pipetted into two tubes and labeled as “Unstained Control” and “CD3/CD19”. Five µL of CD3 - FITC Conjugated Antibody (Beckman Coulter, USA, #A07746), 5 µL of CD19 - ECD Conjugated Antibody (Beckman Coulter, USA, # A07770) were added to the second tube and tube one was used as a negative control. Monoclonal antibodies were obtained from Beckman Coulter, Turkey. Tubes were incubated for 15 minutes at room temperature. After incubation, erythrocyte lysis was performed with 1 mL of VersaLyse Lysing Solution (Beckman Coulter, USA, #A09777) and cells were incubated for 10 minutes at room temperature. Reading was performed on Navios EX Flow Cytometry (Beckman Coulter, USA). The percentage of $CD3^+$ T lymphocytes and $CD19^+$ B lymphocytes on total cells were analyzed with the KALUZA Software Analysis program.

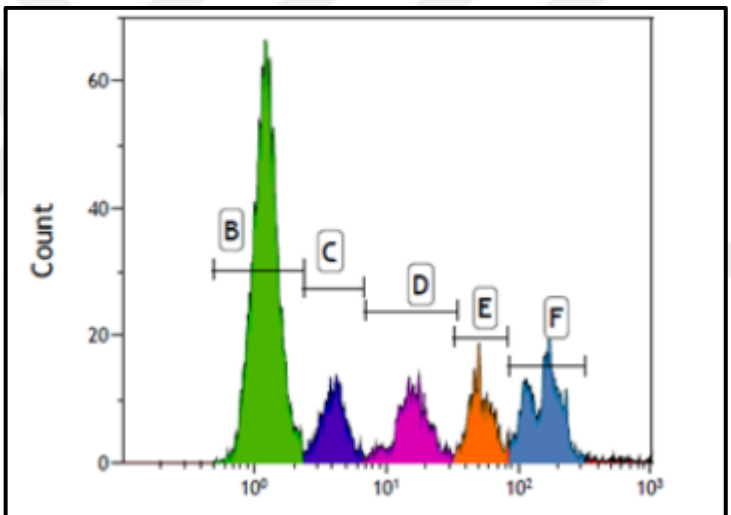
2.2.3. Determination of GM-CSFR Numbers in Apheresis and Peripheral Blood Samples

For determination of GM-CSF receptor numbers, 100 μL of samples were pipetted into two tubes and labeled as "Unstained Control" and "CD45/CD116". Ten μL of CD45 – A750 Conjugated Antibody (Lymphocyte Common Antigen, Beckman Coulter, USA, # A71119), 15 μL of CD116 - PE Conjugated Antibody (Granulocyte-Macrophage Colony-Stimulating Factor Receptor, Beckman Coulter, USA, #IM1977), and 5 μL of CD34 – PC7 Conjugated Antibody (Hematopoietic Progenitor Cell Antigen CD34, Beckman Coulter, USA, #A51077), were added to the second tube and tube one was used as a negative control. Monoclonal antibodies were obtained from Beckman Coulter, Turkey. Tubes were incubated for 15 minutes at room temperature. After incubation, erythrocyte lysis was performed with 1 mL of VersaLyse Lysing Solution (Beckman Coulter, USA, #A09777) and cells were incubated for 10 minutes at room temperature. Reading was performed on Navios EX Flow Cytometry (Beckman Coulter, USA). $CD45^{+}CD116^{+}$ cell absolute number and percentage in lymphocyte, monocyte, granulocyte, and total cells were analyzed with the KALUZA Software Analysis program. MFI represents the intensity of the antibody on the cell surface and correlates with the number of receptors. According to the International Clinical Cytometry Society (ICCS), the MFI of an antigen is calculated by dividing the median of the antigen-positive population by the median of the antigen-negative population [77]. Cells were analyzed according to their size, granularity and the fluorescence intensity of the antibody bound to their surface receptors. All flow cytometry data were reported according to the parameters in the Minimum Information about a Flow Cytometry experiment (MIFlowCyt) checklist [78].

Table 2.1. Antibodies used in flow cytometry analysis

Antibody Name	**Target**	**Conjugated Fluorescence**	**Brand**	**Product No**
CD45/Isotype Control	Lymphocyte Common Antigen	FITC/PE	(Beckman Coulter, USA)	IM3630
CD45/CD34	Lymphocyte Common Antigen/ Hematopoietic Progenitor Cell Antigen CD34	FITC/PE	(Beckman Coulter, USA)	IM3630
7-AAD	DNA	PC5	(Beckman Coulter, USA)	IM3630
CD45	Lymphocyte Common Antigen	A750	(Beckman Coulter, USA)	A71119
CD34	Hematopoietic Progenitor Cell Antigen CD34	PC7	(Beckman Coulter, USA)	A51077
CD116	Granulocyte-Macrophage Colony-Stimulating Factor Receptor	PE	(Beckman Coulter, USA)	IM1977
CD3	T-cell surface Glycoprotein CD3 Precursor	FITC	(Beckman Coulter, USA)	A07746
CD19	B-Lymphocyte Antigen CD19 Precursor	ECD	(Beckman Coulter, USA)	A07770

For the quantitative determination of the GM-CSFR, QIFIKIT Beads (Agilent Dako, USA, #K0078), were analyzed on flow cytometry and calibration was performed. The number of receptors is quantitatively determined by Antigen Binding Capacity (ABC) with fluorescent conjugated beads in QIFIKIT [79]. The kit contains two different bead cocktails: Set-Up Cocktail and Calibration Cocktail. The beads in the Calibration Cocktail have a fluorescent molecule attached to their surface (Figure 2.3.). There are five different bead populations containing 1.900-799.000 receptors on their surface. MFI of each population were matched with related receptor number and ABC were calculated according to Table 2.2. GM-CSF receptor number per cell was compared in apheresis and peripheral blood samples based on QIFIKIT ABC.



Gate	Number	%Gated	X-Med	X-AMean	X-GMean
All	7.039	100	2,46	41,54	6,67
B	3.515	49,9	1,24	1,27	1,23
C	679	9,65	4,07	4,12	4,01
D	863	12,26	16,06	16,73	15,93
E	701	9,96	51,08	53,16	51,92
F	1.198	17,02	161,07	162,25	155,29

Figure 2.3. Analysis of QIFIKIT bead populations

Table 2.2. QIFIKIT analytical values

Calibration Bead	Antibody Molecules Per Bead	MFI	Product No
1	1.900	1,24	K0078
2	8.100	4,07	
3	40.000	16,06	
4	225.000	51,08	
5	799.000	161,07	

MFI: Mean Fluorescence Intensity

Each population carries a different number of antibody molecules. MFI values calculated separately for each population are presented in the table. The number of GM-CSF receptors was calculated in direct proportion by choosing the MFI value close to the MFI of CD116.

2.3. STATISTICAL ANALYSIS

Student's T-Test and One-Way Analysis of Variance (ANOVA) test were utilized for statistical analysis and all statistical analysis was performed by MedCalc version 20.009 software. The P-value less than 0,05 was accepted as statistically significant.

3. RESULTS

3.1. CLASSIFICATION OF STUDY GROUPS

In this study, peripheral blood samples were taken from 20 healthy donors, apheresis samples were taken from 17 autologous donors and 23 allogeneic donors were used. The mean age was 34 (22-47 ± 7,5) for peripheral blood samples, 55 (34-68 ± 10) for autologous donors and 28 (21-46 ± 6,5) for allogeneic donors (Figure 3.1.).

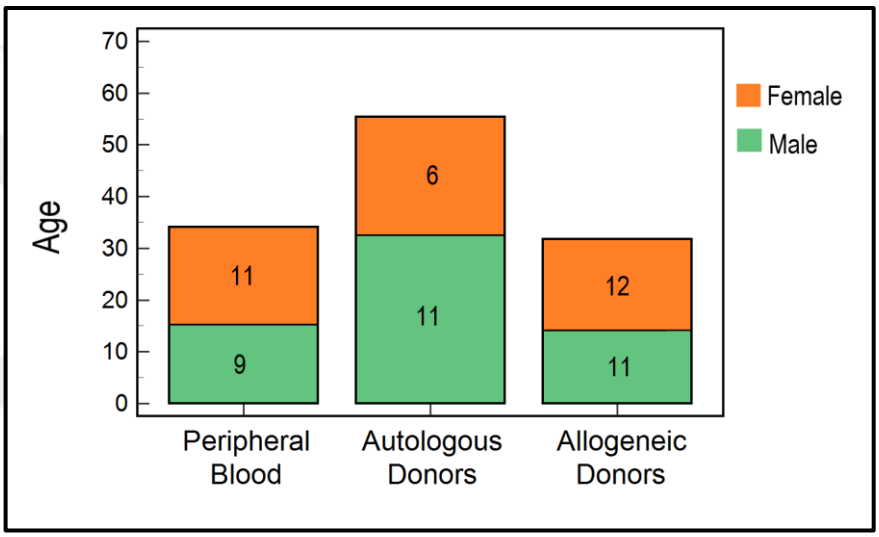


Figure 3.1. Age and gender distribution of peripheral blood controls, autologous and allogeneic donors

Nine of the autologous donors were diagnosed with Non-Hodgkin Lymphoma and eight were diagnosed with Multiple Myeloma. Autologous donors were mobilized with Filgrastim (Neupogen, Amgen Inc, Thousand Oaks, CA) 5 µg/kg/day and allogeneic donors were mobilized with Filgrastim (Neupogen, Amgen Inc, Thousand Oaks, CA) 10 µg/kg/day. Chemotherapy was included in the mobilization regimen of patients with hematological cancer. Patients diagnosed with Non-Hodgkin Lymphoma were mobilized with Etoposide, and patients diagnosed with Multiple Myeloma were mobilized with Cyclophosphamide. Plerixafor was added to the mobilization regimen of four autologous patients. In autologous donors, apheresis was performed by peripheral vascular access in nine patients and by jugular catheterization in eight patients. In autologous donors, apheresis was performed by

peripheral vascular access in 19 patients and by jugular catheterization in four patients. Total blood count was performed on apheresis samples taken from autologous and allogeneic donors (Table 3.1. and Table 3.2.).

Apheresis and peripheral blood WBC in allogeneic donors were found to be approximately 1,5 times higher than apheresis and peripheral blood WBC in autologous donors (Figure 3.2.a and 3.2.b). While the mean WBC count before apheresis was 11.236/μL (1.230 – 23.120 ± 10.450) in autologous donors, the mean WBC count before apheresis was 40.994/μL (21.990 – 65.130 ± 11.183) in allogeneic donors. The mean WBC count in apheresis samples was 124.833/μL (46.500 – 208.200 ± 42.119) in autologous donors, the mean WBC count in apheresis samples was 204.513 (155.800 – 269.700 ± 31.203) in allogeneic donors. The platelet count in allogeneic donors (mean 165 x 10^3/ μL ± 40) was approximately two times higher than the platelet count in autologous donors (mean 79 x 10^3/ μL ± 77) (Figure 3.2.c).

Table 3.1. Total blood count from autologous donors

Donor No	**WBC (Peripheral Blood)**	**WBC (Apheresis)**	**PLT x 10^3**	**Granulocyte (%) (Apheresis)**	**Monocyte (%) (Apheresis)**	**Lymphocyte (%) (Apheresis)**
1	10.170	137.900	20	36,6	34,1	14,5
2	4.540	114.500	25	45,2	9,6	26,4
3	9.510	162.800	59	24,0	14,8	34,7
4	4.470	86.700	87	8,7	31,4	33,5
5	7.180	46.500	166	8,41	30,7	31,0
6	14.910	156.100	352	28,2	27,6	20,4
7	2.720	103.630	30	48,2	22,3	5,2
8	3.200	59.900	61	44,6	15,5	17,4
9	1.230	72.500	38	33,3	17,3	22,5
10	4.470	161.100	80	64,5	15,0	7,6
11	5.460	97.480	91	26,0	32,7	20,3
12	21.460	160.000	92	15,1	59,4	10,8
13	43.240	208.200	29	44,8	30,3	3,9
14	21.280	143.080	56	34,7	25,6	16,6
15	9.200	145.000	26	42,3	23,4	11,0
16	23.120	159.180	43	41,5	11,4	19,5
17	4.860	107.590	95	46,2	21,6	11,3
Mean Values	11.236 ± 10.450	124.833 ± 42.119	79 ± 77	34,8 ± 14,5	24,8 ± 11,4	18,0 ± 9,1

WBC: White Blood Cell, PLT: Platelet

Table 3.2. Total blood count from allogeneic donors

Donor No	**WBC (Peripheral Blood)**	**WBC (Apheresis)**	**PLT x 10^3**	**Granulocyte (%) (Apheresis)**	**Monocyte (%) (Apheresis)**	**Lymphocyte (%) (Apheresis)**
1	21.990	178.100	107	37,4	34,2	12,6
2	29.210	161.000	216	34,4	23,7	23,7
3	32.970	155.300	205	33,0	18,2	30,3
4	35.280	210.300	172	20,9	26,7	35,4
5	39.890	187.400	178	40,3	21,2	25,1
6	31.890	171.800	168	26,8	27,8	25,5
7	65.130	230.100	161	43,1	17,5	16,6
8	49.190	192.900	171	37,2	23,2	25,3
9	33.090	204.300	196	47,7	23,2	13,5
10	50.280	249.100	142	15,4	32,8	35,1
11	46.030	269.700	173	40,7	20,8	17,7
12	55.580	233.200	57	12,0	32,5	39,9
13	53.700	223.800	163	42,3	18,7	22,3
14	43.550	211.800	133	36,2	16,6	26,9
15	29.320	238.300	129	36,0	17,3	31,6
16	35.630	199.000	160	24,3	25,0	31,2
17	43.120	228.100	243	38,5	20,8	23,8
18	47.160	172.800	204	25,6	21,7	42,0
19	47.580	155.800	214	20,8	37,0	29,2
20	40.070	241.400	164	41,0	20,0	26,0
21	52.050	223.000	162	38,5	21,6	18,3
22	24.260	183.400	147	23,2	26,6	36,7
23	34.300	183.200	168	38,9	21,4	23,5
Mean Values	40.994 ± 11.183	204.513 ± 31.203	165 ± 40	32,8 ± 9,4	23,8 ± 5,6	26,6 ± 5,8

WBC: White Blood Cell, PLT: Platelet

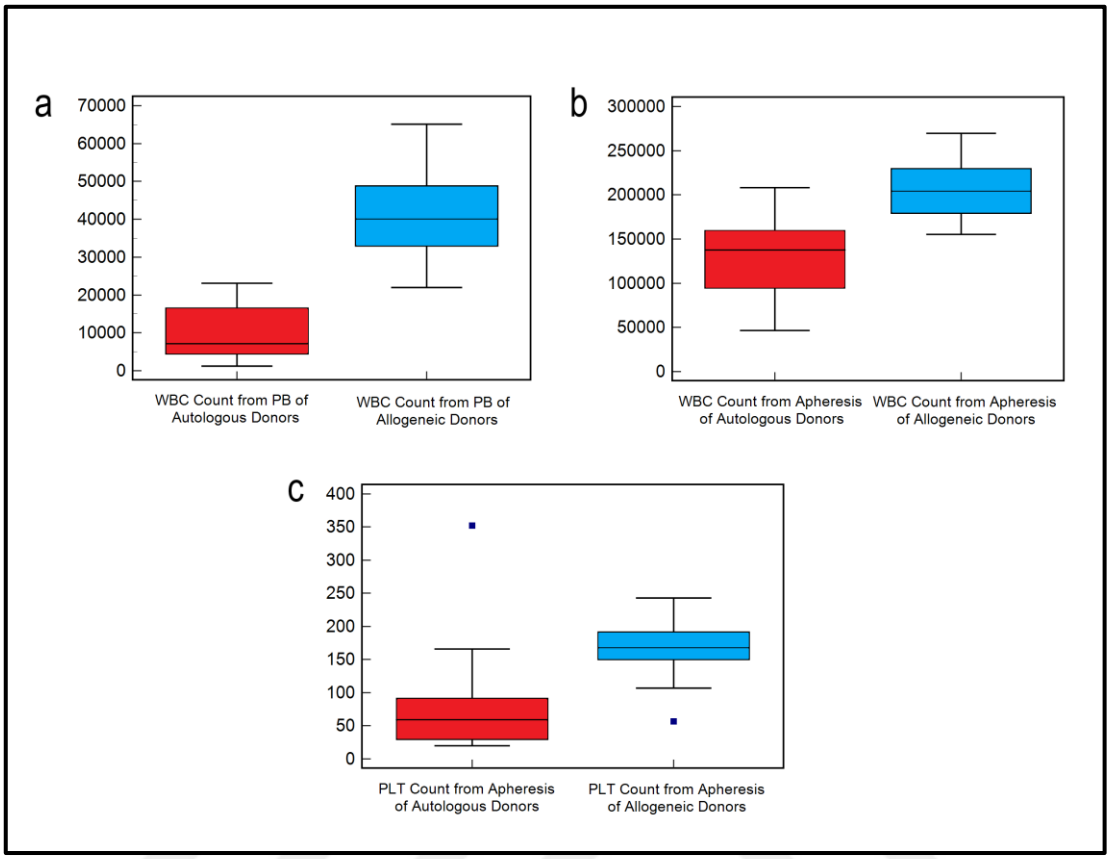


Figure 3.2. Comparison of WBC and PLT counts between donors. (a) Comparison of WBC counts in peripheral blood samples from autologous and allogeneic donors, (b) Comparison of WBC counts in apheresis samples from autologous and allogeneic donors, (c) Comparison of PLT counts in apheresis samples from autologous and allogeneic donors ($p < 0{,}0001$)

3.2. EVALUATION OF CD34$^+$ STEM CELL COUNTS IN COLLECTED APHERESIS SAMPLES

The percentage and the absolute number of CD34$^+$ cells in the peripheral blood before collection and the post-collection apheresis sample were determined by flow cytometry. In autologous donors, the mean percentage of CD34$^+$ cells in peripheral blood was 1,01 percent (0,1 – 3,77 ± 1,03) and the mean absolute CD34$^+$ cell number was 78/µL (7 – 582 ± 133). The mean percentage of CD34$^+$ cells in apheresis samples was 1,46 percent (0,21 – 5,65 ± 1,23) and the mean absolute CD34$^+$ cell number was 1.743/µL (272 – 7.752 ± 1.871) (Table 3.3.).

Table 3.3. CD34$^+$ cell count from autologous donors

Donor No	Apheresis			Peripheral Blood	
	Percentage of CD45$^+$ Cells	Percentage of CD34$^+$ Cells	Absolute Number of CD34$^+$ Cells/µL	Percentage of CD34$^+$ Cells	Absolute Number of CD34$^+$ Cells/µL
1	99,5	0,21	272	0,1	9
2	99,0	4,90	4.858	3,77	145
3	99,4	0,96	1.305	0,6	51
4	98,8	5,1	2.436	2,74	85
5	95,6	1,15	279	0,15	5
6	99,0	1,9	2.117	0,82	92
7	98,9	1,35	1.231	1,09	19
8	92,8	1,04	312	0,56	7
9	97,9	1,02	534	0,94	7
10	99,0	1,2	1.719	0,88	29
11	99,8	1,22	802	0,39	15
12	99,8	0,74	1.013	0,12	23
13	99,9	0,54	1.037	0,25	102
14	99,7	2,01	2.548	0,69	131
15	99,6	0,34	428	0,13	8
16	99,7	5,65	7.752	2,74	582
17	98,4	1,43	1.000	1,35	26
Mean Values	98,6 ± 1,7	1,46 ± 1,23	1.743 ± 1.871	1,01 ± 1,03	78 ± 133

CD34: Hematopoietic Progenitor Cell Antigen CD34, CD45: Lymphocyte Common Antigen

In allogeneic donors, the mean percentage of $CD34^+$ cells in peripheral blood was 0,24 percent (0,13 – 0,38 ± 0,06) and the mean absolute $CD34^+$ cell number was 90/µL (25 – 147 ± 36). The mean percentage of $CD34^+$ cells in apheresis samples was 0,85 percent (0,46 – 1,34 ± 0,26) and the mean absolute $CD34^+$ cell number was 1.461/µL (713 – 2.670 ± 491) (Table 3.4.).

Table 3.4. $CD34^+$ cell count from allogeneic donors

Donor No	Apheresis			Peripheral Blood	
	Percentage of $CD45^+$ Cells	Percentage of $CD34^+$ Cells	Absolute Number of $CD34^+$ Cells/µL	Percentage of $CD34^+$ Cells	Absolute Number of $CD34^+$ Cells/µL
1	99,8	0,46	713	0,13	25
2	99,5	1,34	1.585	0,34	87
3	99,0	0,61	813	0,23	58
4	99,2	0,76	1.329	0,21	68
5	99,7	1,04	1.738	0,33	120
6	99,0	0,74	1.051	0,2	56
7	99,3	1,04	2.160	0,29	179
8	99,6	0,66	1.101	0,15	72
9	98,9	0,5	936	0,17	52
10	99,4	0,75	1.759	0,25	121
11	99,4	0,68	1.731	0,22	99
12	99,4	1,0	2.108	0,24	128
13	99,3	0,53	1.099	0,24	115
14	98,4	0,92	1.580	0,28	97
15	99,0	0,74	1.679	0,26	74
16	99,1	0,74	1.298	0,17	56
17	99,1	0,92	1.955	0,26	109
18	99,3	1,22	1.918	0,26	116
19	98,8	0,96	1.314	0,24	106
20	99,5	1,2	2.670	0,38	147
21	99,3	0,5	1.031	0,14	71
22	99,5	0,53	803	0,14	31
23	99,9	0,82	1.240	0,34	105
Mean Values	99,2 ± 0,3	0,85 ± 0,26	1.461 ± 491	0,24 ± 0,06	90 ± 36

CD34: Hematopoietic Progenitor Cell Antigen CD34, CD45: Lymphocyte Common Antigen

Cells labeled with PC7-conjugated CD34 antibody were analyzed in the FL-5 channel and cells labeled with A750-conjugated CD45 antibody were analyzed in the FL-8 channel (Figure 3.3.).

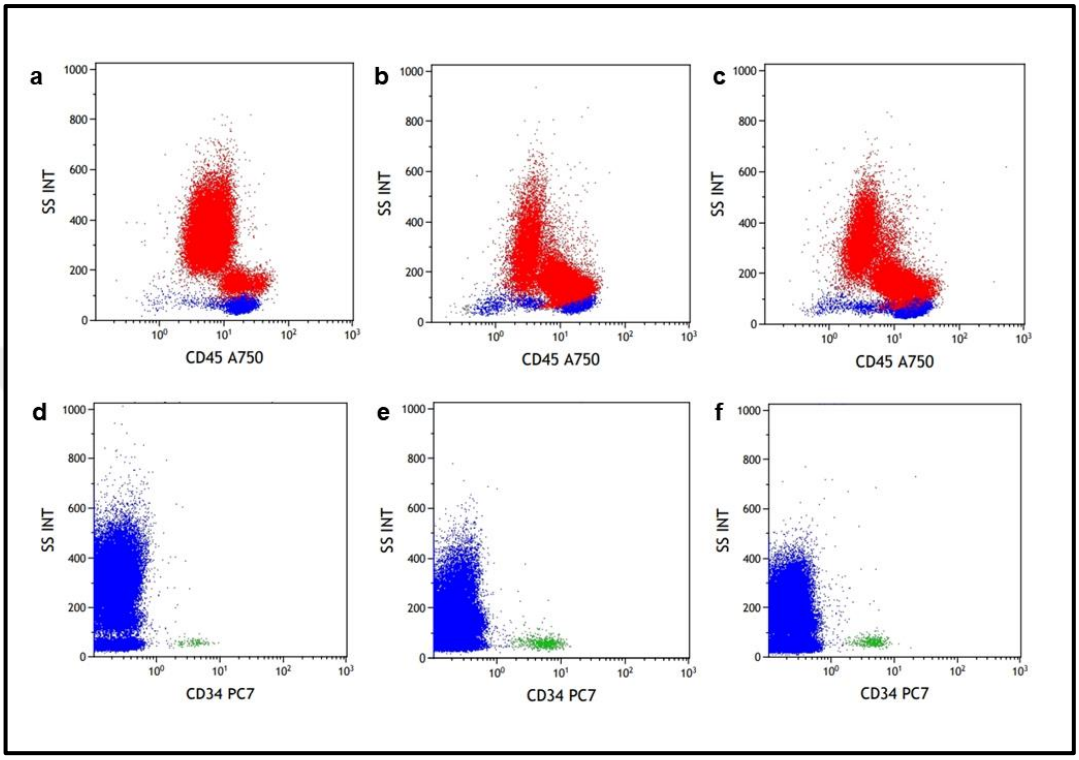


Figure 3.3. Stem cell characterization in peripheral blood and apheresis samples. (a) $CD45^+$ leukocytes in peripheral blood, (b) $CD45^+$ leukocytes in an apheresis sample of an autologous donor, (c) $CD45^+$ leukocytes in an apheresis sample of an allogeneic donor, d) $CD34^+$ stem cells in peripheral blood, (e) $CD34^+$ stem cells in an apheresis sample of an autologous donor, (f) $CD34^+$ stem cells in an apheresis sample of an allogeneic donor

As expected, the percentage of $CD45^+$ cells was above 95 percent in all samples. It was found that the percentage of CD34 in the peripheral blood of autologous donors was approximately four times higher than the percentage of CD34 in the peripheral blood of allogeneic donors (Figure 3.4.a). Similarly, the percentage of CD34 in apheresis samples from autologous donors was approximately 2-fold higher than the percentage of CD34 in apheresis samples from allogeneic donors (Figure 3.4.b). When the absolute $CD34^+$ cell count in peripheral blood was examined, it was found that the absolute $CD34^+$ cell count in allogeneic donors was approximately 1,15 times higher than in autologous donors (Figure 3.4.c). When the absolute $CD34^+$ cell count in apheresis samples was compared, it was found

that the absolute $CD34^+$ cell count in autologous donors was 1,19 times higher than in allogeneic donors (Figure 3.4.d).

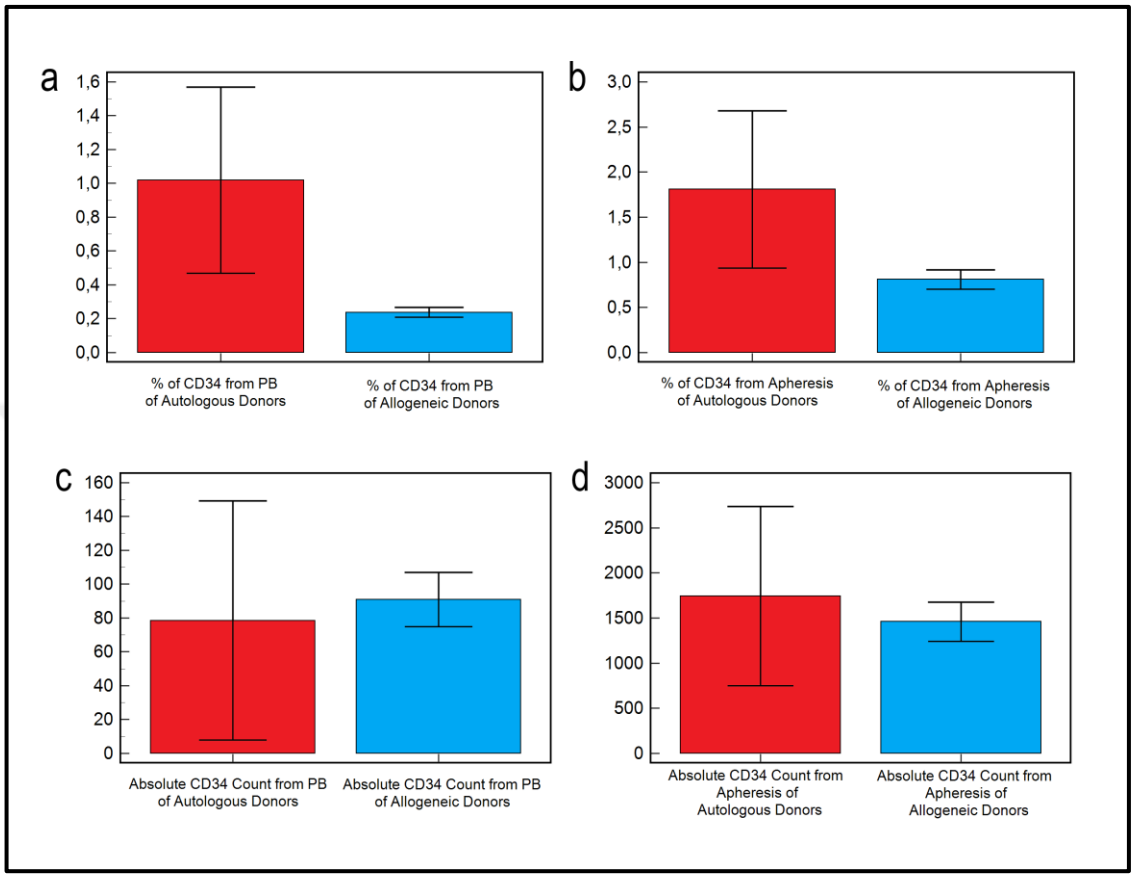


Figure 3.4. Comparison of CD34 percentage and absolute cell counts in peripheral blood and apheresis samples. (a) Comparison of $CD34^+$ cell percentage in peripheral blood samples from autologous and allogeneic donors ($p = 0,0012$) , (b) Comparison of $CD34^+$ cell percentage in apheresis samples from autologous and allogeneic donors ($p = 0,0081$), (c) Comparison of absolute $CD34^+$ cell counts in peripheral blood samples from autologous and allogeneic donors ($p < 0,001$), (d) Comparison of absolute $CD34^+$ cell counts in apheresis samples from autologous and allogeneic donors ($p < 0,001$)

The reason for the higher error bar in mean CD34 percentage and absolute count in peripheral blood and apheresis samples from autologous donors is due to the heterogeneity of the patient group. Although not significant, we found that Multiple Myeloma patients had a higher $CD34^+$ cell percentage and absolute count, so we concluded that mobilization of Multiple Myeloma patients was more successful than the mobilization of Lymphoma patients.

In both autologous and allogeneic donors, the estimated number of $CD34^+$ cells determined by calculating the $CD34^+$ cell yield significantly correlated with the number of collected $CD34^+$ cells (Figure 3.5.a and 3.5.b). The collection efficiency coefficient was calculated separately for autologous and allogeneic donors. While the collection efficiency was 52,3 percent in allogeneic donors, it was 84,4 percent in autologous donors. Collection efficiency in autologous donors was higher than collection efficiency in allogeneic donors (Figure 3.5.c).

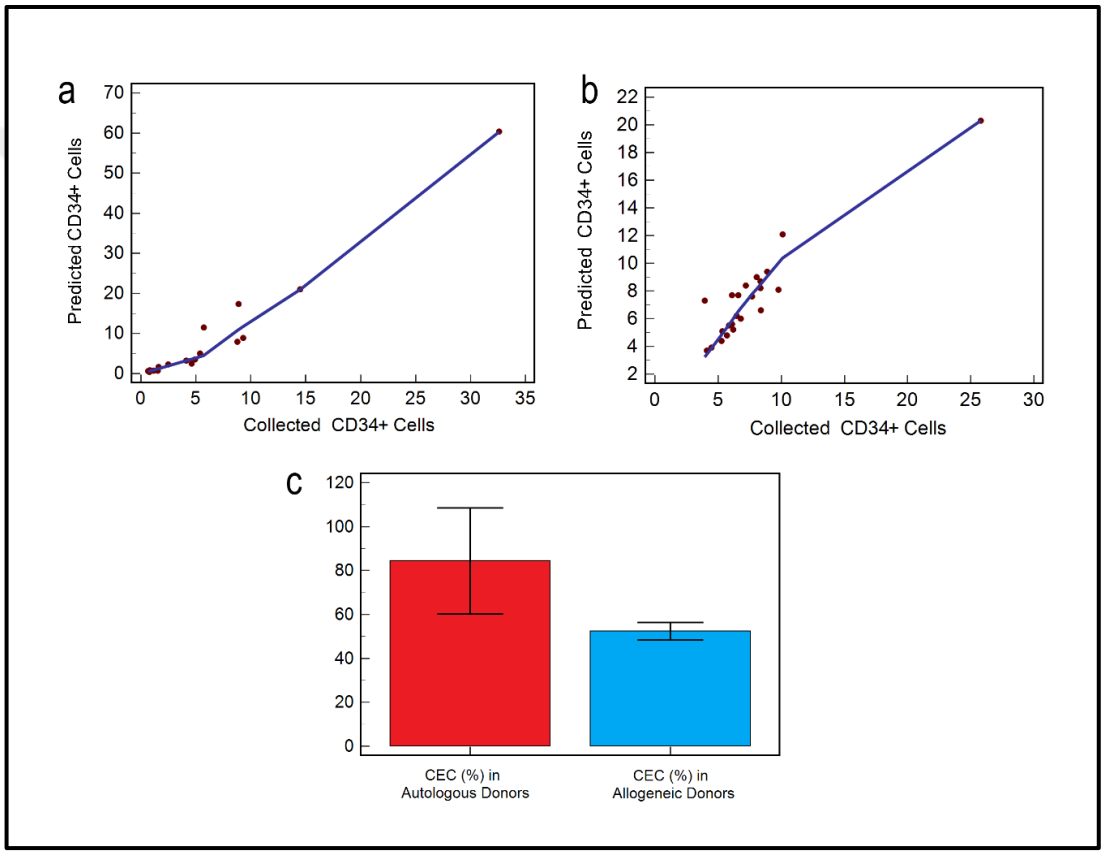


Figure 3.5. Predicted and collected $CD34^+$ cell counts and efficiency. (a) Predicted versus collected $CD34^+$ cells in autologous donors ($p < 0,0001$), (b) Predicted versus collected $CD34^+$ cells in allogeneic donors ($p < 0,0001$), (c) Comparison of collection efficiency coefficient in autologous and allogeneic donors ($p = 0,002$)

3.3. GM-CSFR NUMBER IN AUTOLOGOUS DONORS

Cells labeled with PE-conjugated CD116 antibody were analyzed in the FL-2 channel. (Figure 3.6.). In the analysis performed on total cells, the mean $CD116^+$ percentage was found to be 71,4 percent (48,0 – 92,3 ± 12,6). Co-staining was performed for CD34 and CD116 and we found that $CD34^+$ cells did not express CD116 (Figure 3.6.c).

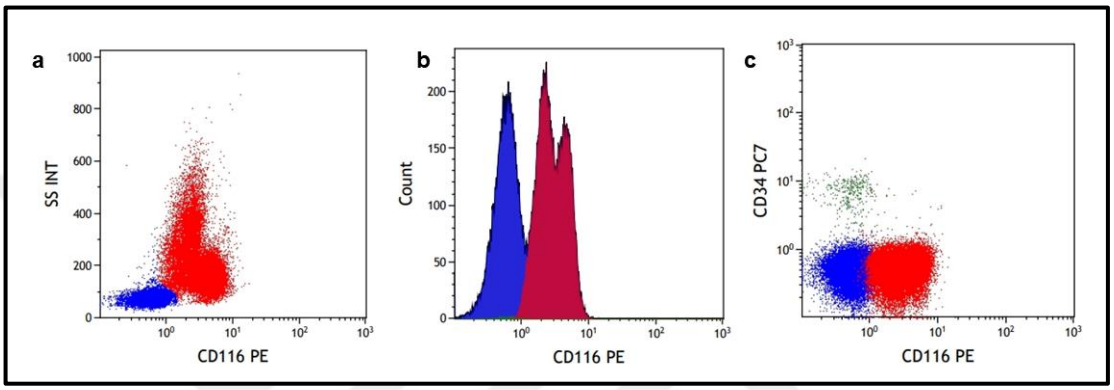


Figure 3.6. Expression of CD116 in apheresis sample of an autologous donor. (a) $CD116^+$ myeloid cells, (b) Histogram for $CD116^+$ cells, (c) $CD34^+CD116^+$ cells

CD116 percentage was evaluated on lymphocytes, monocytes, granulocytes and total cells, but since there was no significant result on lymphocytes (mean 0,6 percent ± 0,4), analysis was performed only with monocytes, granulocytes and total cells (Table 3.5.).

Table 3.5. Percentage of CD116 on different cell groups in autologous donors

Donor No	Lymphocytes	Monocytes	Granulocytes	Total
1	1,19	98,9	99,5	80,1
2	0,42	99,4	99,6	63,9
3	1,35	96,1	99,9	54,5
4	0,91	99,7	99,9	51,8
5	0,21	99,7	99,5	48,0
6	1,24	99,3	99,6	72,7
7	0,84	98,8	99,9	85,4
8	0,92	96,2	99,2	66,4
9	0,28	98,8	100	60,9
10	0,26	97,6	100	86,3
11	0,2	97,0	98,8	66,0
12	0,44	99,6	99,5	82,9
13	0,99	99,5	99,9	92,3
14	0,28	98,8	99,3	72,3
15	1,3	99,1	99,8	81,8
16	0,3	98,9	99,6	68,4
17	0,2	99,0	99,6	80,7
Mean Values	0,6 ± 0,4	98,6 ± 1,12	99,6 ± 0,3	71,4 ± 12,6

The mean percentage of CD116 on monocytes was 98,6 percent (96,1 – 99,7 ± 1,12) and the mean percentage of CD116 on granulocytes was 99,6 percent (98,8 – 100 ± 0,3). The mean percentage of CD116 on granulocytes was higher than on monocytes (Figure 3.7.a). When the fluorescence intensity was examined, the MFI of CD116 on monocytes was 4,6 (2,51 - 7,6 ± 1,2), while the MFI of CD116 on granulocytes was 3,07 (2,43 – 4,75 ± 0,6). Total MFI of CD116 was 3,46 (2,18 – 6,22 ± 1,0). When the GM-CSFR number was calculated, an average of 9.155 ABC/Cell (3.845 – 15.125 ± 26.200) receptors were found on monocytes and an average of 5.800 ABC/Cell (3.723 - 9.453 ± 1.634) receptors were found on granulocytes. The number of receptors on total cells was 6.632 ABC/Cell (3.340 - 12.378 ± 2.447). MFI of CD116 and GM-CSFR number on monocytes were higher than on granulocytes (Figure 3.7.b and 3.7.c) (Table 3.6.).

Table 3.6. MFI of CD116 and GM-CSFR number on myeloid cells in autologous donors

Donor No	MFI of CD116			GM-CSFR Number (ABC/Cell)		
	Monocytes	**Granulocytes**	**Total**	**Monocytes**	**Granulocytes**	**Total**
1	4,11	2,97	3,23	8.179	5.910	6.428
2	4,27	2,81	2,47	8.498	5.592	3.784
3	5,64	2,9	3,96	11.224	5.771	7.881
4	6,46	3,98	5,95	12.856	7.920	11.841
5	5,18	2,7	2,58	10.309	5.373	3.953
6	3,22	2,88	3,11	6.408	5.731	6.189
7	4,46	3,2	2,81	8.876	6.368	5.592
8	4,64	2,66	2,18	9.234	5.293	3.340
9	7,6	4,23	3,32	15.125	8.418	6.607
10	4,53	4,75	3,39	9.015	9.453	6.746
11	4,7	2,55	3,77	9.353	3.907	7.502
12	6,3	3,14	6,22	12.538	6.249	12.378
13	3,6	3,26	3,54	7.164	7.045	7.045
14	3,75	2,43	3,12	7.463	3.723	6.209
15	4,47	2,53	4,0	8.896	3.876	7.960
16	3,35	2,64	2,39	6.667	4.045	3.662
17	2,51	2,57	2,83	3.845	3.937	5.632
Mean Values	4,6 ± 1,2	3,07 ± 0,6	3,46 ± 1,0	9.155 ± 2.620	5.800 ± 1.634	6.632 ± 2.447

MFI: Mean Fluorescence Intensity, GM-CSFR: Granulocyte-Macrophage Colony-Stimulating Factor Receptor, ABC: Antigen Binding Capacity

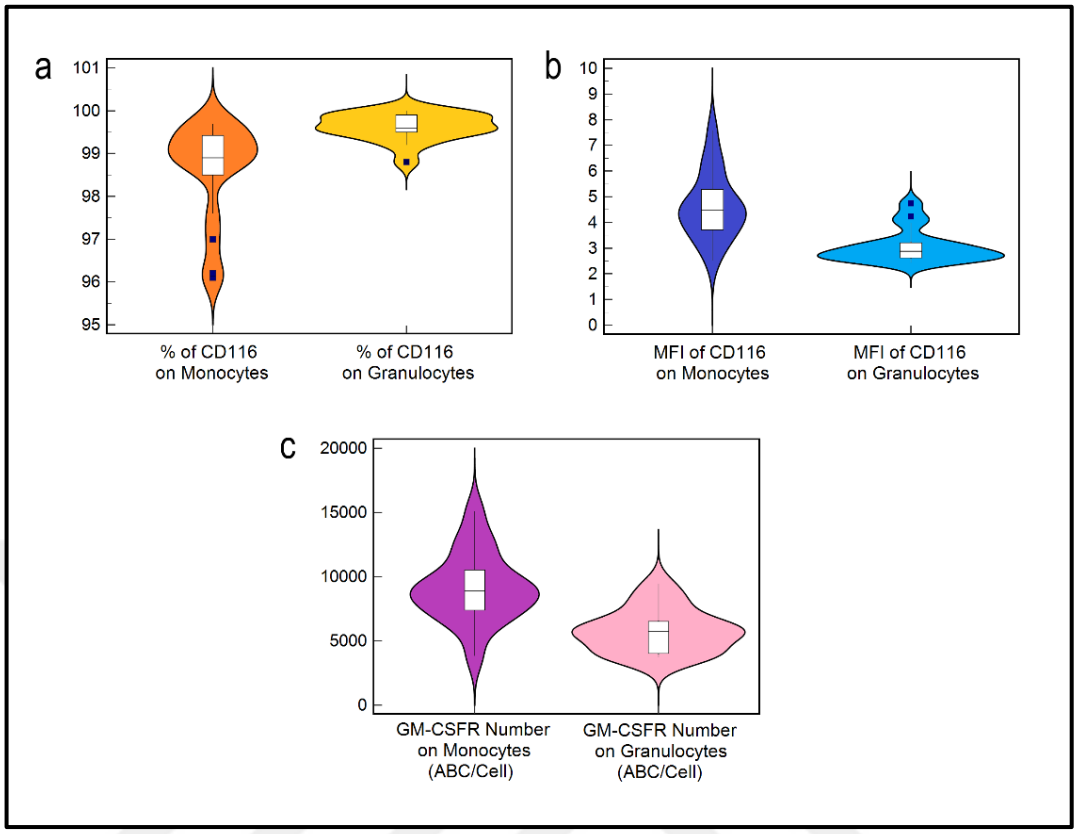


Figure 3.7. Expression of CD116 and GM-CSFR numbers on myeloid cells in autologous donors. (a) Percentage of CD116 on monocytes and granulocytes ($p = 0,0018$), (b) MFI of CD116 on monocytes and granulocytes ($p < 0,0001$), (c) GM-CSFR numbers on monocytes and granulocytes ($p < 0,0001$)

The GM-CSFR number and CD116 MFI were negatively correlated with weight (Figure 3.8.a and 3.8.b) and there was no significant correlation between GM-CSFR and age, gender or height in autologous donors. CD116 percentage was positively correlated with leukocyte count in apheresis samples (Figure 3.8.c) while there was no significant relation between CD116 percentage and peripheral blood WBC count. When GM-CSFR number, percentage and MFI value are compared with CD34 percentage and absolute number in peripheral blood and apheresis samples, no significant correlation was observed. There was also no significant correlation between GM-CSFR and platelet count, viability, G-CSF dose, the type of chemotherapy, collected total CD34 cells, CD34 yield and collection efficiency coefficient. We also compared collected CD34 cells with age, gender, height, weight peripheral blood and apheresis WBC, platelet count but observed no correlation. In autologous donors, chemotherapy type, indication and administration of Plerixafor (Mozobil, Genzyme

Corporation, Cambridge, MA) had no effect on the GM-CSFR and the number of collected $CD34^+$ stem cells.

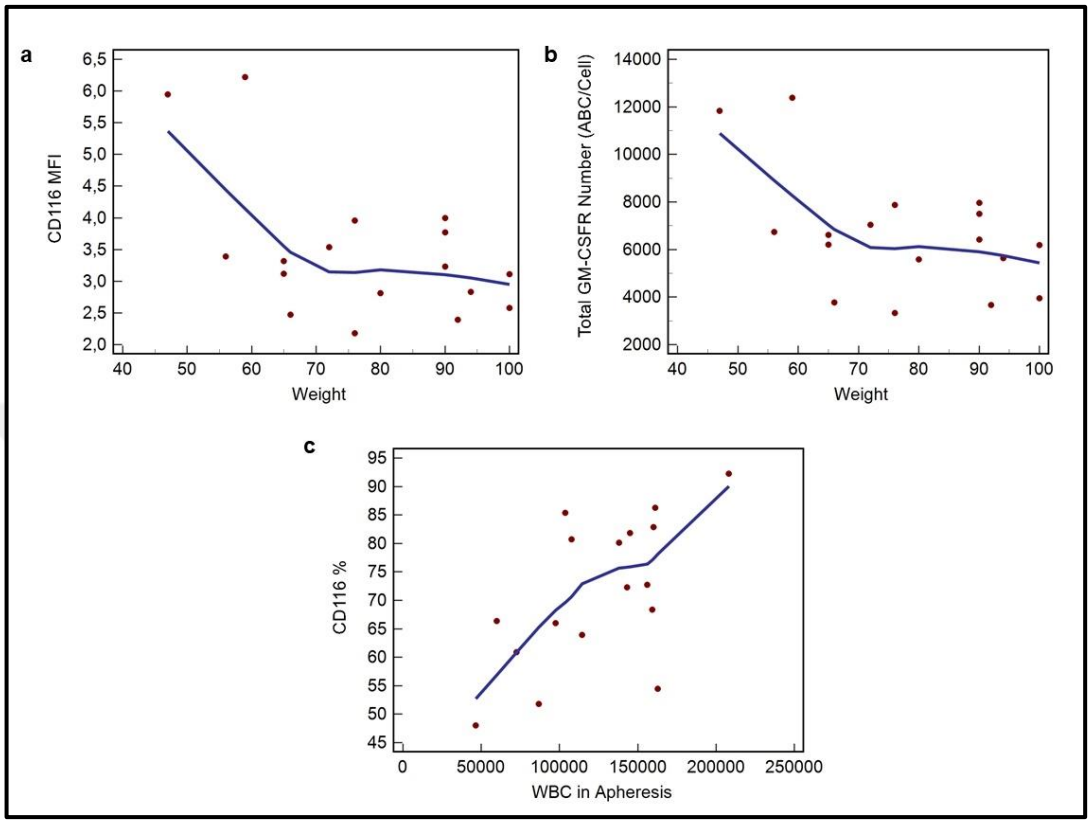


Figure 3.8. Correlation of CD116 percentage, MFI and GM-CSFR number in autologous donors. (a) Correlation of CD116 MFI with weight of donors (p = 0,03), (b) Correlation of GM-CSFR number with weight of donors (p = 0,02), (c) Correlation of CD116 percentage with WBC in apheresis samples of autologous donors (p = 0,008)

3.4. GM-CSFR NUMBER IN ALLOGENEIC DONORS

Cells labeled with PE-conjugated CD116 antibody were analyzed in the FL-2 channel. (Figure 3.9.). In the analysis performed on total cells, the mean $CD116^+$ percentage was found to be 67,3 percent (51,8 – 83,5 ± 8,3). Co-staining was performed for CD34 and CD116 and we found that $CD34^+$ cells did not express CD116 (Figure 3.9.c).

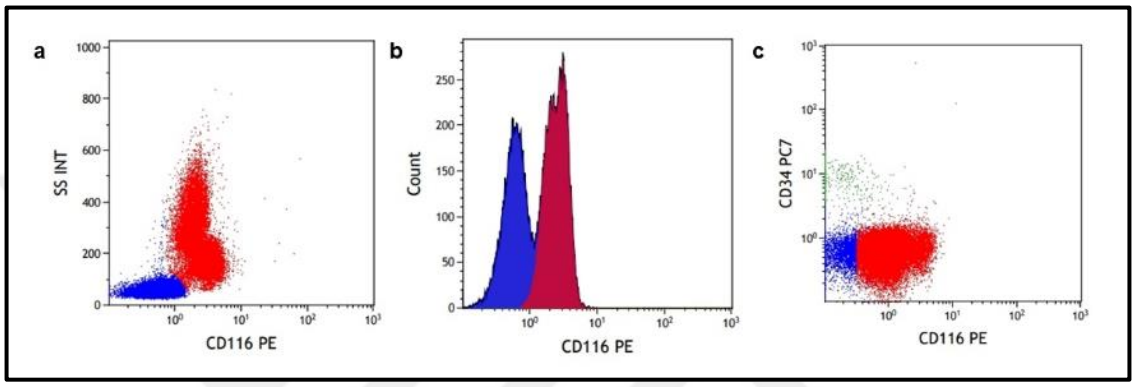


Figure 3.9. Expression of CD116 in apheresis sample of an allogeneic donor. (a) $CD116^+$ myeloid cells, (b) Histogram for $CD116^+$ cells, (c) $CD34^+CD116^+$ cells

Similar to autologous donors, the percentage of CD116 was evaluated on lymphocytes, monocytes, granulocytes, and total cells, but as there was no significant result on lymphocytes (mean 0,1 percent ± 0,1), analyze was performed with monocytes, granulocytes, and total cells only (Table 3.7.).

Table 3.7. Percentage of CD116 on different cell groups in allogeneic donors

Donor No	Lymphocytes	Monocytes	Granulocytes	Total
1	0,22	99,4	99,9	83,5
2	0,23	99,5	99,5	68,6
3	0,17	99,8	99,6	63,9
4	0,24	99,5	99,3	56,5
5	0,26	99,5	99,9	69,5
6	0,24	99,6	100	65,4
7	0,06	99,1	99,7	74,1
8	0,03	99,6	99,9	68,9
9	0,37	99,7	99,8	82,6
10	0,32	99,8	99,9	59,4
11	0,02	99,5	99,9	76,4
12	0,05	99,7	99,8	51,8
13	0,04	99,4	99,9	73,7
14	0,02	98,7	99,8	65,4
15	0,07	99,5	99,8	63,5
16	0,11	99,7	99,9	62,1
17	0,12	99,5	99,9	70,2
18	0,2	99,9	99,9	52,5
19	0,17	99,9	99,8	66,0
20	0,05	99,6	99,9	69,0
21	0,0	99,5	99,6	76,1
22	0,2	99,7	99,8	57,3
23	0,2	99,8	99,7	72,1
Mean Values	0,1 ± 0,1	99,5 ± 0,2	99,7 ± 0,1	67,3 ± 8,3

The mean percentage of CD116 on monocytes was 99,5 percent (98,7 – 99,9 ± 0,2) and the mean percentage of CD116 on granulocytes was 99,7 percent (99,3 – 100 ± 0,1) (Figure 3.10.a). When the fluorescence intensity was examined, the MFI of CD116 on monocytes was 5,0 (3,13 – 8,09 ± 1,2), while the MFI of CD116 on granulocytes was 3,1 (2,56 – 4,1 ± 0,4). Total MFI of CD116 was 3,79 (2,55 – 4,95 ± 0,7). When the GM-CSFR number was calculated, an average of 9.965 ABC/Cell (6.229 – 16.100 ± 2.479) receptors were found on monocytes and an average of 6.290 ABC/Cell (3.968 – 8.159 ± 908) receptors were found

on granulocytes. The number of receptors on total cells was 7.506 ABC/Cell (3.907 – 11.304 ± 1.540). MFI of CD116 and GM-CSFR number on monocytes were higher than on granulocytes (Figure 3.10.b and 3.10.c) (Table 3.8.).

Table 3.8. MFI of CD116 and GM-CSFR number on myeloid cells in allogeneic donors

Donor No	**MFI of CD116**			**GM-CSFR Number (ABC/Cell)**		
	Monocytes	**Granulocytes**	**Total**	**Monocytes**	**Granulocytes**	**Total**
1	5,58	3,42	4,43	11.105	6.806	8.816
2	5,68	2,77	3,46	11.304	5.512	6.885
3	4,43	2,88	3,32	8.816	5.731	6.607
4	4,5	2,59	3,71	8.955	3.968	7.383
5	5,75	3,05	3,64	11.443	6.070	7.244
6	8,09	4,05	5,68	16.100	8.060	11.304
7	4,7	2,9	3,96	9.353	5.771	7.881
8	4,09	3,18	4,64	8.139	6.328	9.234
9	4,93	3,19	3,29	9.811	6.348	6.547
10	5,63	3,75	4,95	11.204	7.463	9.851
11	3,97	3,18	3,54	7.900	6.328	7.045
12	3,13	3,6	2,55	6.229	7.164	3.907
13	5,71	3,56	4,42	11.363	7.085	8.796
14	4,4	2,81	3,01	8.756	5.592	5.990
15	3,92	3,23	3,43	7.801	6.428	6.826
16	4,97	3,32	4,04	9.891	6.607	8.040
17	7,89	4,1	4,6	15.702	8.159	9.154
18	5,34	3,18	3,22	10.627	6.328	6.408
19	6,47	2,93	4,17	12.876	5.831	8.299
20	3,65	3,15	3,27	7.264	6.269	6.507
21	3,31	2,86	2,76	6.587	5.691	5.492
22	5,04	3,04	3,61	10.030	6.050	7.184
23	4,0	2,56	3,64	7.960	5.094	7.244
Mean Values	5,0 ± 1,2	3,1 ± 0,4	3,79 ± 0,7	9.965 ± 2.479	6.290 ± 908	7.506 ± 1.540

MFI: Mean Fluorescence Intensity, GM-CSFR: Granulocyte-Macrophage Colony-Stimulating Factor Receptor, ABC: Antigen Binding Capacity

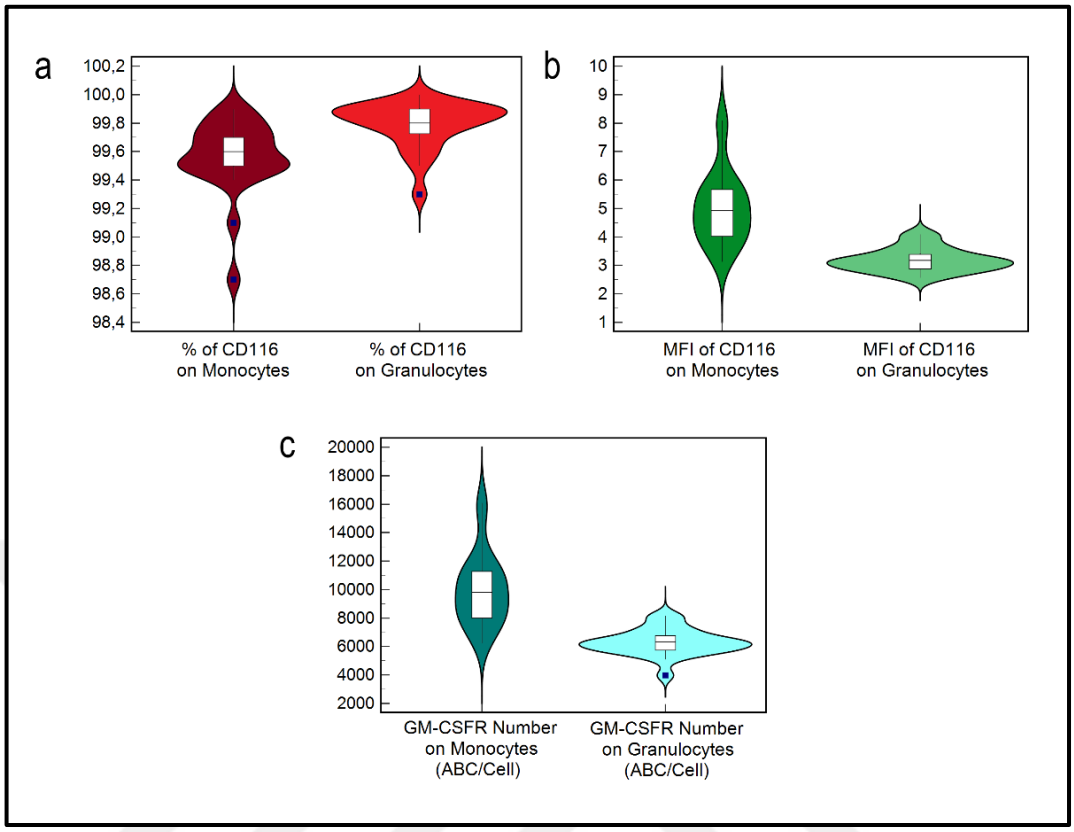


Figure 3.10. Expression of CD116 and GM-CSFR numbers on myeloid cells in allogeneic donors. (a) Percentage of CD116 on monocytes and granulocytes ($p = 0,0012$), (b) MFI of CD116 on monocytes and granulocytes ($p < 0,0001$), (c) GM-CSFR numbers on monocytes and granulocytes ($p < 0,0001$)

While the CD116 percentage was positively correlated with age, no correlation was observed with weight, height and gender in allogeneic donors (Figure 3.11.a). The relationship between CD116 percentage and B and T lymphocytes was examined. The percentage of CD116 was found to have a negative correlation with the percentage of $CD3^+$ T lymphocytes and $CD19^+$ B lymphocytes (Figure 3.11.b and 3.11.c). GM-CSFR number, percentage and MFI value compared with CD34 percentage and absolute number in peripheral blood and apheresis sample, viability, G-CSF dose, collected total CD34 cells, CD34 yield and collection efficiency coefficient but no significant correlation was observed. We also found that collected total $CD34^+$ cells were significantly correlated with platelet count (Figure 3.11.d).

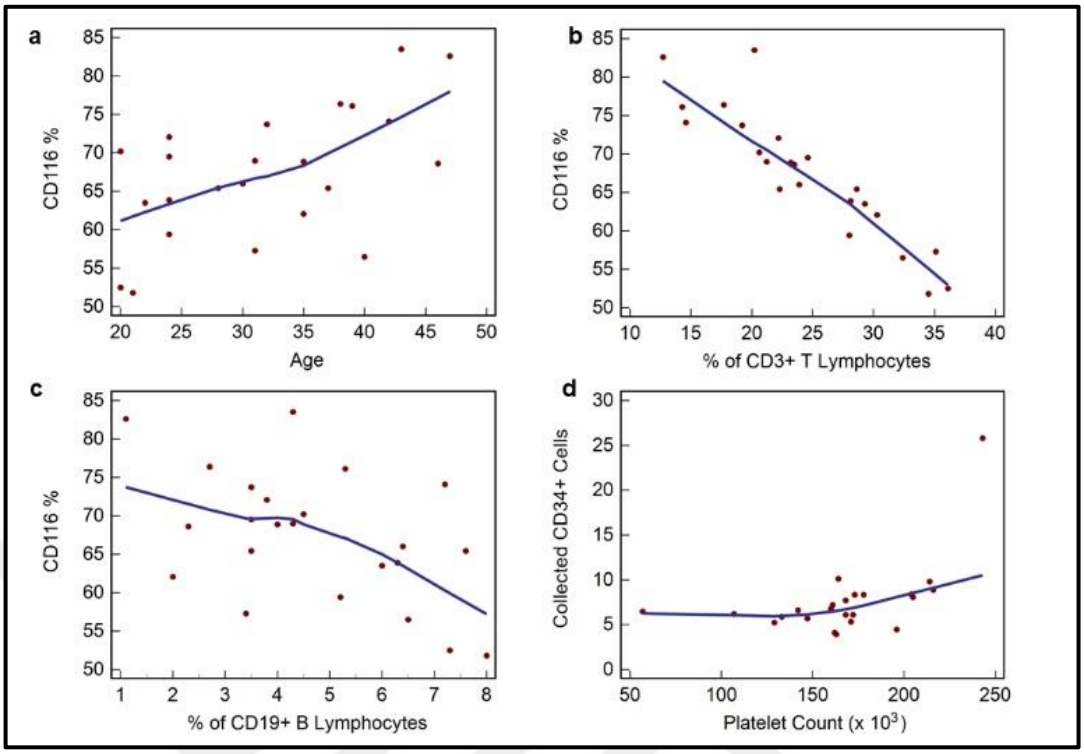


Figure 3.11. Correlation of CD116 percentage with T and B lymphocytes, collected $CD34^+$ cells and platelet count in allogeneic donors. (a) Correlation of CD116 percentage with age in apheresis samples of allogeneic donors (p = 0,004), (b) Correlation of CD116 percentage with $CD3^+$ T lymphocytes in apheresis samples of allogeneic donors (p < 0,0001), (c) Correlation of CD116 percentage with $CD19^+$ B lymphocytes in apheresis samples of allogeneic donors (p = 0,01), (d) Correlation of collected $CD34^+$ cells with platelet count in apheresis samples of allogeneic donors (p = 0,009)

3.5. GM-CSFR NUMBER IN PERIPHERAL BLOOD

Cells labeled with PE-conjugated CD116 antibody were analyzed in the FL-2 channel. (Figure 3.12.). In the analysis performed on total cells, the mean $CD116^+$ percentage was found to be 64,3 percent (53,4 – 74,3 ± 5,5). Co-staining was performed for CD34 and CD116 and we found that $CD34^+$ cells did not express CD116 (Figure 3.12.c).

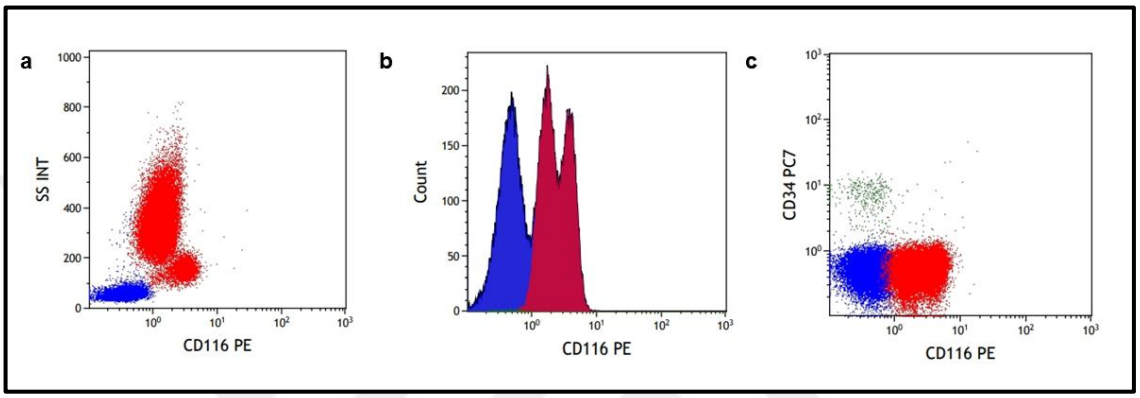


Figure 3.12. Expression of CD116 in peripheral blood. (a) $CD116^+$ myeloid cells, (b) Histogram for $CD116^+$ cells, (c) $CD34^+CD116^+$ cells

CD116 percentage was evaluated on lymphocytes, monocytes, granulocytes and total cells, but since there was no significant result on lymphocytes (mean 0,1 percent ± 0,08), analysis was performed only with monocytes, granulocytes and total cells (Table 3.9.).

Table 3.9. Percentage of CD116 on different cell groups in peripheral blood

Donor No	Lymphocytes	Monocytes	Granulocytes	Total
1	0,05	99,6	100	68,9
2	0,1	98,9	100	58,7
3	0,16	99,3	100	68,0
4	0,08	99,8	100	53,4
5	0,14	99,9	99,7	64,0
6	0,02	99,5	100	64,3
7	0,04	99,3	99,9	69,4
8	0,08	99,4	99,9	63,3
9	0,04	99,4	99,9	60,1
10	0,13	99,6	99,9	66,2
11	0,13	98,9	99,9	58,3
12	0,08	99,3	99,8	64,5
13	0,33	99,7	99,9	65,0
14	0,2	99,9	99,9	58,9
15	0,07	99,2	100	60,5
16	0,34	99,9	99,7	74,3
17	0,16	99,4	99,9	78,0
18	0,05	98,7	99,8	67,5
19	0,09	99,6	100	62,7
20	0,23	97,5	100	60,9
Mean Values	0,1 ± 0,08	99,3 ± 0,5	99,9 ± 0,09	64,3 ± 5,5

The mean percentage of CD116 on monocytes was 99,3 percent (97,5 – 99,9 ± 0,5) and the mean percentage of CD116 on granulocytes was 99,9 percent (99,7 – 100 ± 0,09) (Figure 3.13.a). When the fluorescence intensity was examined, the MFI of CD116 on monocytes was 10,8 (6,3 – 23,2 ± 4,4), while the MFI of CD116 on granulocytes was 3,0 (1,8 – 4,58 ± 0,7). Total MFI of CD116 was 3,82 (2,71 – 7,59 ± 1,13). When the GM-CSFR number was calculated, an average of 24.013 ABC/Cell (12.538 – 57.783, ± 12.585) receptors were found on monocytes and an average of 5.864 ABC/Cell (2.758 – 9.114 ± 1.705) receptors were found on granulocytes. The number of receptors on total cells was 7.618 ABC/Cell (5.393 – 15.105 ± 2.251). MFI of CD116 on monocytes was approximately 3,6 times higher than the MFI of CD116 on granulocytes (Figure 3.13.b). The number of GM-CSFR on monocytes

was approximately 4 times higher than the number of GM-CSFR on granulocytes. (Figure 3.13.c) (Table 3.10.).

Table 3.10. MFI of CD116 and GM-CSFR number on myeloid cells in peripheral blood

Donor No	**MFI of CD116**			**GM-CSFR Number (ABC/Cell)**		
	Monocytes	**Granulocytes**	**Total**	**Monocytes**	**Granulocytes**	**Total**
1	9,31	2,36	3,17	18.528	3.616	6.308
2	8,83	2,9	4,28	17.573	5.771	8.517
3	8,3	4,58	3,31	16.518	9.114	6.587
4	9,42	2,41	3,17	18.747	4.796	6.308
5	6,3	2,1	4,13	12.538	3.217	8.219
6	9,25	4,44	3,91	18.409	8.836	7.781
7	8,63	3,19	2,9	17.175	6.348	5.771
8	8,18	3,05	3,17	16.279	6.070	6.308
9	8,05	3,2	3,25	16.020	6.368	6.468
10	8,31	2,87	3,9	16.538	5.711	7.761
11	9,7	3,43	3,28	19.304	6.826	6.527
12	8,82	2,92	2,71	17.553	5.811	5.393
13	13,6	2,86	4,01	33.872	5.691	7.980
14	10,1	2,78	3,94	25.155	5.532	7.841
15	19,2	4,41	7,59	47.820	8.776	15.105
16	19,43	3,02	2,78	48.393	6.010	5.532
17	8,31	3,3	3,64	16.538	6.567	7.244
18	7,88	2,97	3,04	15.682	5.910	6.050
19	23,2	2,32	6,0	57.783	3.554	11.941
20	11,98	1,8	4,39	29.838	2.758	8.736
Mean Values	10,8 ± 4,4	3,0 ± 0,7	3,82 ± 1,13	24.013 ± 12.585	5.864 ± 1.705	7.618 ± 2.251

MFI: Mean Fluorescence Intensity, GM-CSFR: Granulocyte-Macrophage Colony-Stimulating Factor Receptor, ABC: Antigen Binding Capacity

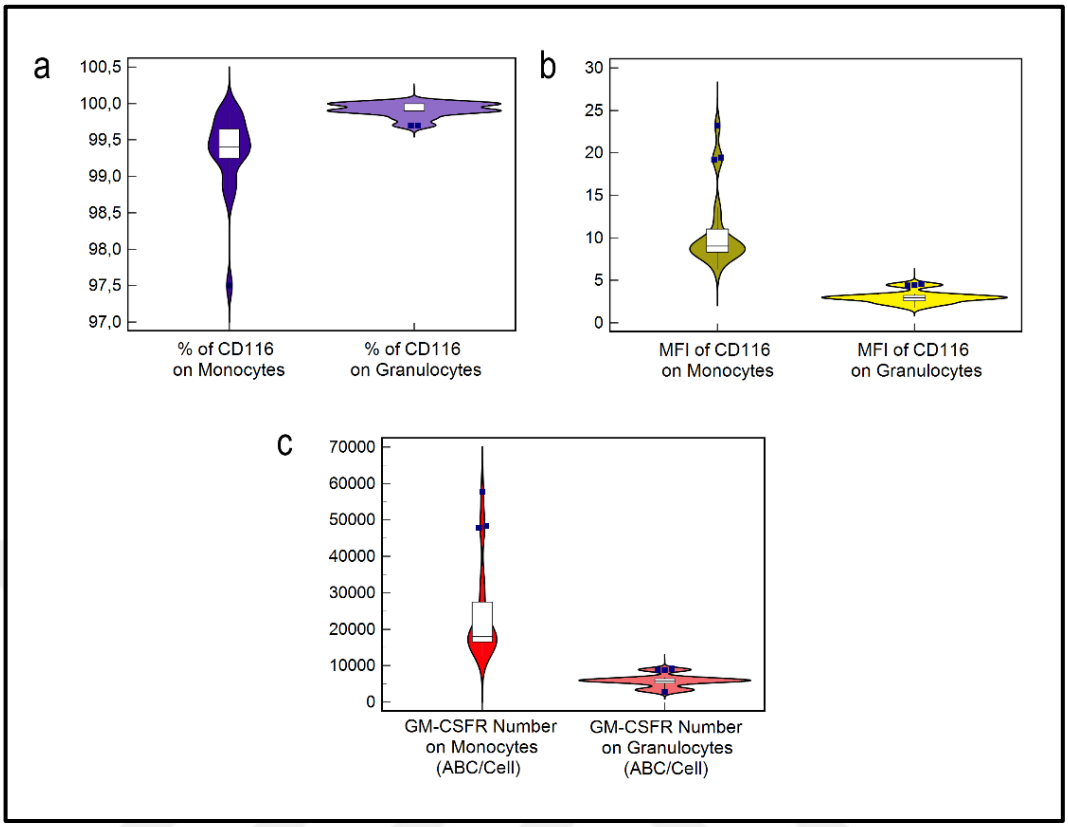


Figure 3.13. Expression of CD116 and GM-CSFR numbers on myeloid cells in peripheral blood. (a) Percentage of CD116 on monocytes and granulocytes ($p = 0{,}0003$), (b) MFI of CD116 on monocytes and granulocytes ($p < 0{,}0001$), (c) GM-CSFR numbers on monocytes and granulocytes ($p < 0{,}0001$)

In the peripheral blood of healthy volunteers not administered G-CSF, no correlation was observed between CD116 percentage, MFI, and GM-CSFR number and age, gender, and WBC count.

3.6. COMPARISON OF AUTOLOGOUS AND ALLOGENEIC DONATIONS AND PERIPHERAL BLOOD

After determining the percentage and MFI of CD116 and GM-CSFR numbers in apheresis samples from autologous and allogeneic donors and peripheral blood samples from healthy volunteers, comparisons between groups were made (Table 3.11.).

Table 3.11. Mean values of MFI of CD116 and GM-CSFR numbers in apheresis samples from autologous and allogeneic donors and peripheral blood samples

	GM-CSFR Number (ABC/Cell)			**MFI of CD116**		
	Total	**Monocytes**	**Granulocytes**	**Total**	**Monocytes**	**Granulocytes**
Peripheral Blood (n=20)	7.618 ± 2.251	24.013 ± 12.585	5.864 ± 1.705	3,8 ± 1,13	10,8 ± 4,4	3,04 ± 0,7
Autologous Donors (n=17)	6.632 ± 2.447	9.155 ± 2.620	5.800 ± 1.634	3,46 ± 1,0	4,6 ± 1,2	3,07 ± 0,6
Allogeneic Donors (n=23)	7.506 ± 1.540	9.965 ± 2.479	6.690 ± 908	3,79 ± 0,7	5,0 ± 1,2	3,1 ± 0,4

MFI: Mean Fluorescence Intensity, GM-CSFR: Granulocyte-Macrophage Colony-Stimulating Factor Receptor, ABC: Antigen Binding Capacity

In autologous and allogeneic donors, the number of GM-CSFR and MFI of CD116 on monocytes are approximately 1,5 times higher than the number of receptors and MFI of CD116 on granulocytes. Since the most significant result was seen in monocytes, we investigated the number of GM-CSFRs on monocytes between groups. The number of GM-CSFR in monocytes was compared and the highest expression was seen in peripheral blood with the number of an average of 24.013 receptors. When compared between donations, the highest GM-CSFR number on monocytes was detected in allogeneic donors with an average of 9.965 receptors, followed by autologous donors with an average of 9.155 receptors (Figure 3.14.a). MFI of CD116 on monocytes was compared and the highest MFI was

detected in peripheral blood with an average of 10,8. When compared between donations, the highest MFI of CD116 on monocytes was detected in allogeneic donors with an average of 5,0 followed by autologous donors with an average of 4,6 (Figure 3.14.c). Unlike monocytes, no significant results were obtained when comparing the number of GM-CSFR and MFI of CD116 on granulocytes between groups (Figure 3.14.b and 3.14.d).

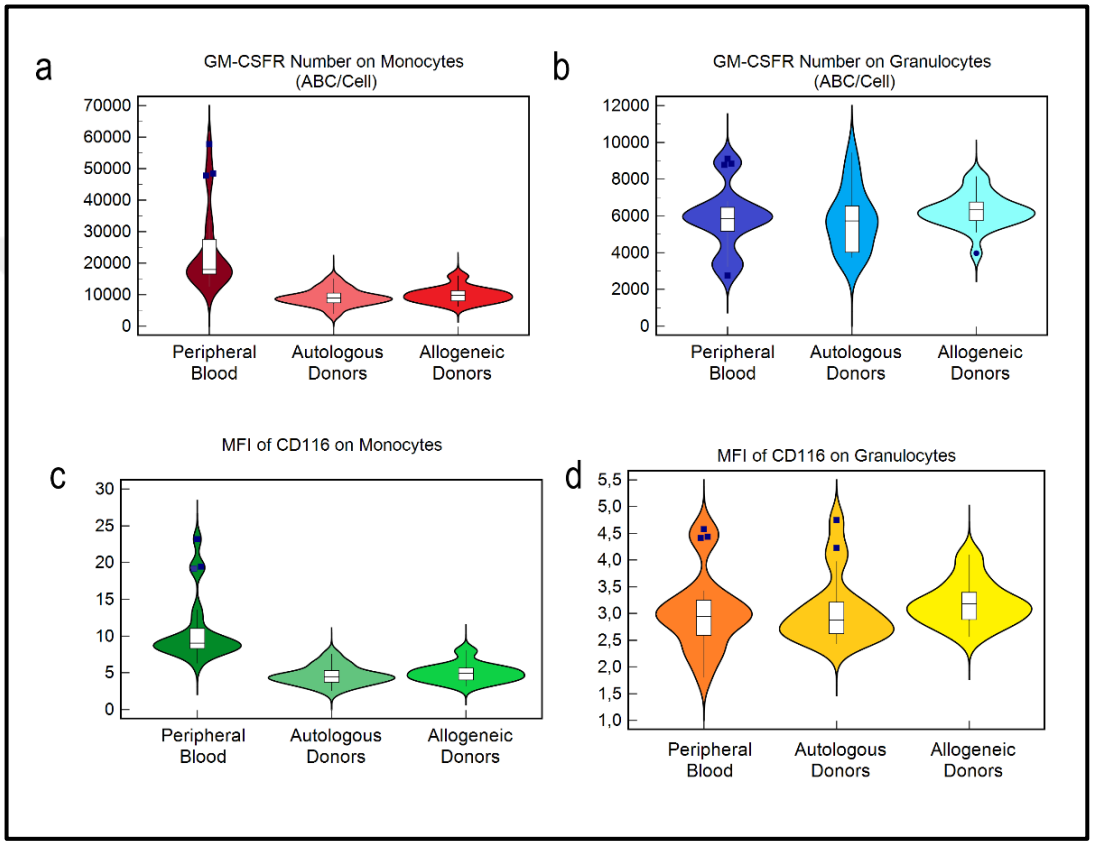


Figure 3.14. Comparison of GM-CSFR number and MFI of CD116 between groups. (a) Comparison of GM-CSFR number on monocytes between groups (p = 0,0006), (b) Comparison of GM-CSFR number on granulocytes between groups (p = 0,5), (c) Comparison of MFI of CD116 on monocytes between groups (p = 0,0001), (d) Comparison of MFI of CD116 on granulocytes between groups (p = 0,5)

When the number of GM-CSF receptors in monocytes and MFI of CD116 were examined, a more uniform distribution was observed in apheresis samples from autologous and allogeneic donors compared to peripheral blood. The number of GM-CSF receptors and MFI of CD116 in granulocytes had a similar density in all groups.

As shown in Figure 3.15., the percentage and MFI of CD116 and GM-CSFR numbers on total cells were compared between groups, but no significant results were obtained, consistent with similar density in violin plots.

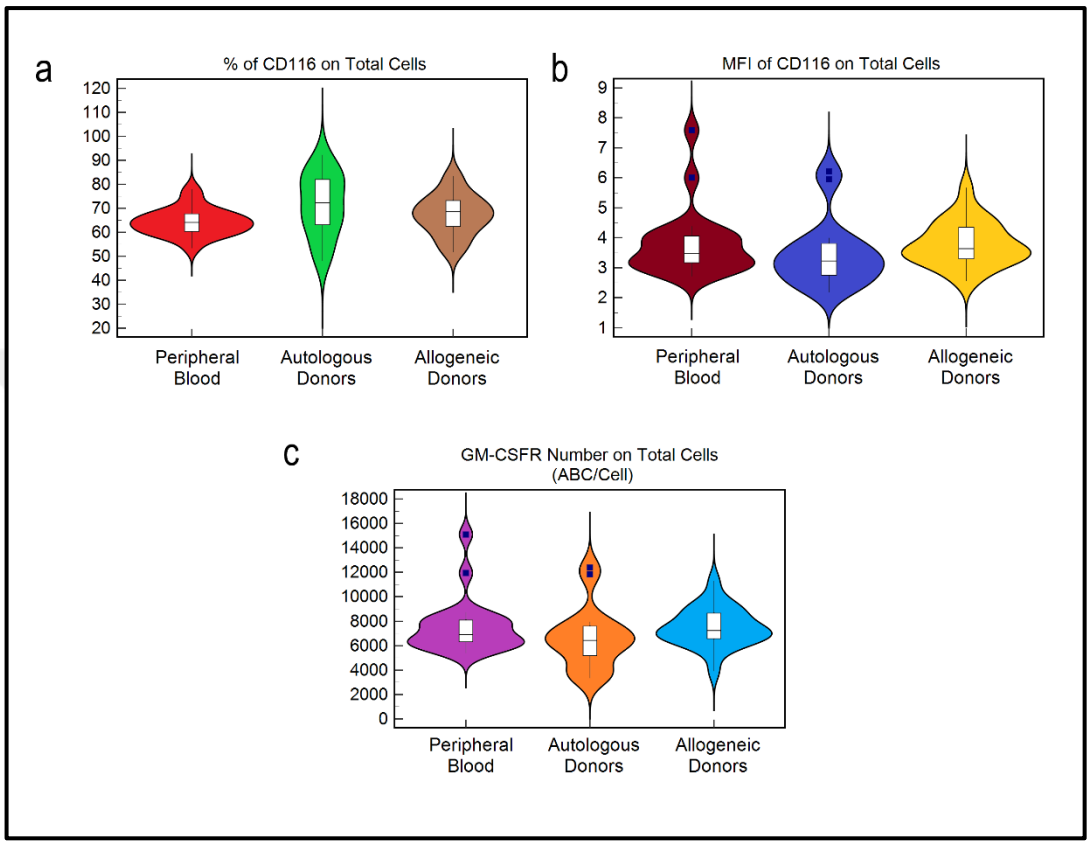


Figure 3.15. Comparison between groups in analysis on total cells. (a) Comparison of percentage of CD116 on total cells, (b) Comparison of MFI of CD116 on total cells, (d) Comparison of GM-CSFR number on total cells (p = 0,6)

We have reached to below conclusions by observation of these results:

- No significant difference was observed when the percentage and MFI of CD116 and GM-CSFR numbers on all cells were compared, but interesting differences were detected between groups and between cells.
- The highest MFI of CD116 and GM-CSFR numbers were detected on monocytes in all groups.
- When the groups were compared, the highest MFI of CD116 and GM-CSFR numbers were detected in peripheral blood.
- No significant correlation was found between CD116 and other predictive factors, but the highest MFI of CD116 and GM-CSFR numbers were detected in apheresis samples from allogeneic donors with higher WBC and PLT counts than autologous donors.
- As a result, we concluded that there is an indirect relationship between CD116 and the WBC and PLT counts and that CD116 may be useful in evaluating the quality of apheresis samples.

4. DISCUSSION

Stem cell transplantation is considered as a promising method in the treatment of many diseases, especially hematological diseases [34]. Nowadays, the most used cell sources for stem cell transplantations are bone marrow, peripheral stem cells and cord blood, and the cell source is decided by considering criteria such as the type of disease, prognosis and age of the patient [80]. Nowadays, peripheral stem cell collection with the apheresis method, which has been used since the 20th century, is preferred because it does not require surgery and hospitalization. Compared with bone marrow, transplantation with peripheral stem cells has a higher risk of acute and chronic GVHD, and its effect on survival rate is not superior to bone marrow [80,81]. Therefore, evaluation of the quality of the apheresis product is critical for successful transplantation and engraftment follow-up. Although $CD34^+$ cell enumeration is considered as the gold standard in determining the quality of the apheresis product, the optimum $CD34^+$ cell percentage and number varies in each center and does not provide information about the myeloid cells in the apheresis product [45]. In recent years, granulocyte transfusion has been used in the treatment of bacterial and fungal infections and GVHD due to stem cell transplantation [82]. Therefore, in addition to $CD34^+$ cells, determining the proportion and number of myeloid cells in the apheresis product can predict transplant success or even prevent post-transplant adverse effects.

The GM-CSF receptor (CD116) was chosen as the myeloid cell marker because CD116 is expressed in all mature granulocyte and monocyte cells starting from the myeloid cell progenitors [3]. In this study, the percentage of $CD45^+CD116^+$ cells, MFI of CD116 and GM-CSF receptor number were determined and compared. In addition, the relationship of CD116 with other parameters was examined. In the study of Lanza et al., it was stated that normal $CD34^+$ cells in the bone marrow express CD116, but in our study, no significant $CD34^+CD116^+$ cell population was detected in healthy peripheral stem cells [71]. This difference can be explained by the fact that stem cells resident in the bone marrow are hematopoietic progenitor stem cells at an earlier stage than peripheral stem cells stimulated with G-CSF.

To investigate whether stimulation with G-CSF has an effect on CD116 expression, peripheral blood samples from healthy donors who did not receive G-CSF and apheresis samples from autologous and allogeneic donors were compared. When the percentage and MFI of total CD116, and number of GM-CSF receptors in autologous and allogeneic donors and peripheral blood were compared, no significant difference was detected. From this point of view, myeloid cell groups were compared and the percentage of CD116 did not show any significant difference both between the groups and between cells, whereas the MFI of CD116 and GM-CSF receptor numbers were higher on monocytes compared to granulocytes. Monocytes were examined and the highest GM-CSF receptor number and MFI of CD116 were detected in peripheral blood followed by allogeneic donors and lowest in autologous donors (Figure 3.14.a and 3.14.c). Lee et al. reported that the MFI of CD116 and the number of GM-CSF receptors in the peripheral blood were highest on classical monocytes expressing high levels of HLA-DR, followed by intermediate monocytes and lowest on non-classic monocytes [73]. Since classical monocytes are known to constitute approximately 90 percent of the monocyte subsets in peripheral blood, the highest CD116 expression on monocytes may be due to the high percentage of classical monocytes in peripheral blood [72]. When the number of MFI and GM-CSF receptors on granulocytes were compared, no significant difference was observed between the groups. Since the expression of the GM-CSF receptor is associated with maturation, similar GM-CSF receptor levels in each group are expected in mature granulocytes [2].

In autologous and allogeneic donors, no significant correlation was found between the percentage and MFI of CD116 and the GM-CSF receptor number, and the percentage and number of $CD34^+$ cells collected. For this reason, we investigated the correlation of CD116 expression with other parameters. The correlation of CD116 expression with other factors in apheresis samples from autologous and allogeneic donors differed from each other. MFI of CD116 and GM-CSF receptor numbers were negatively correlated with weight in autologous donors, while weight was not correlated with MFI of CD116 and GM-CSF receptor numbers in allogeneic donors. The percentage of total CD116 in apheresis samples from autologous donors was significantly correlated with apheresis WBC count ($p= 0,008$). Based on these data, we detected a higher WBC count in apheresis samples containing a high percentage of CD116. These results correlated with higher peripheral blood and apheresis WBC counts and PLT counts in allogeneic donors that expressed higher CD116 compared to autologous

donors. The percentage of CD116 in allogeneic donors was positively correlated with age. Considering that the mean age of allogeneic donors is lower than that of autologous donors, we can conclude that CD116 is expressed higher in young donors. When the percentage of CD116 and the percentage of B and T lymphocytes in allogeneic donors were examined, it was seen that there was a negative correlation. (Figure 3.11.b and 3.11.c). GM-CSF is produced by B and T lymphocytes under different conditions, but the GM-CSF receptor is not expressed on these cells [83].

Autologous donors mobilized with G-CSF and chemotherapy showed faster cell growth than donors mobilized with G-CSF alone [48]. However, as expected, the WBC and PLT counts produced in autologous donors after mobilization with chemotherapy + G-CSF are lower than in healthy allogeneic donors (Figure 3.2.). This is due to the regeneration of the bone marrow, which is completely suppressed by chemotherapy [84]. The dose of G-CSF for both groups of donors, type of chemotherapy and inclusion of Plerixafor in the mobilization regimen in autologous donors did not affect CD116 expression. According to the study by Corso et al., in autologous donors, the PLT count in the apheresis product correlates with the number of $CD34^+$ cells collected [85]. In this study, there was no significant correlation between PLT count and the number of $CD34^+$ cells collected in apheresis samples from autologous donors, while a significant correlation was found between PLT count and $CD34^+$ cell count collected in apheresis samples from allogeneic donors ($p = 0,009$). Calculation of $CD34^+$ cell yield and Collection Efficiency Coefficient before apheresis provides more effective cell collection. The optimal Collection Efficiency Coefficient for allogeneic donors is in the range of 30 to 50 percent, and many factors can change this range [76]. The higher Collection Efficiency Coefficient detection in autologous donors than in allogeneic donors is due to the initiation of the collection while a lower percentage of $CD34^+$ cells are circulating in the peripheral blood in autologous donors. This finding indicates that the number of $CD34^+$ cells in peripheral blood changes the percentage of Collection Efficiency Coefficient, as reported by Tiwari et al [86]. In autologous and allogeneic donors, $CD34^+$ cell yield and the number of collected $CD34^+$ cells have a significant correlation, a result of successful stem cell collection ($p < 0,0001$).

5. CONCLUSION

Although the beginning of peripheral stem cell transplantation dates back to the 20th century, it is frequently used in the treatment of hematological diseases in many countries and health centers today. Since cell collection is a vital process for both the donor and the patient, it is crucial to determine the quality of the collected cells, to determine the optimum cell amount for transplantation and to follow the engraftment process after transplantation. Currently, only CD34 and CD45 markers are used in the flow cytometric evaluation of the apheresis product. In this study, we used CD116 as a marker to evaluate the quality of apheresis products in autologous and allogeneic donors for the first time. As a result of this study, we determined that percentage of CD116 did not differ between both groups and on myeloid cells. When MFI of CD116 and GM-CSFR number were compared, it was found that CD116 was most intensely expressed in allogeneic donors, and most intensely expressed on monocytes when cells were compared. Although there was no statistically direct correlation, a higher WBC and PLT count was obtained in allogeneic donors expressing high MFI of CD116 and GM-CSFR receptor number, and a direct correlation was found between the percentage of CD116 in autologous donors and the number of WBCs in the apheresis product. Finally, we conclude that CD116 may be used as a marker in assessing the quality of the apheresis product and further experimentation is required for its use as a predictive indicator for stem cell transplantation.

6. FUTURE DIRECTIONS

In apheresis samples, in addition to the fact that CD116 expression at the protein level was determined by flow cytometry, detection of CD116 gene expression by quantitative Polymerase Chain Reaction (q-PCR) and mRNA expression by Real-Time Polymerase Chain Reaction (RT-PCR) will support the understanding of the dynamics of this receptor.

Since G-CSF increases both cell number and maturation, it should be investigated to what extent the expression of G-CSF receptor (CD114) changes in apheresis products and what kind of relationship it has with CD116.

Since sufficient numbers of autologous and allogeneic donors could not be collected due to the COVID-19 pandemic, more sample examinations will increase the accuracy of the results.

Finally, examining the effects of CD116 expression in apheresis samples on long-term engraftment and GVHD may contribute to the importance of evaluating CD116 in apheresis samples.

REFERENCES

1. Hoglund M. Hematopoietic effects and clinical application of granulopoietic growth factors. *Ups J Med Sci.* 1996;101(2):121–48.
2. Metcalf D. The molecular control of cell division, differentiation commitment and maturation in haemopoietic cells. *Nature.* 1989;339(6219):27–30.
3. Alberts B, Johnson A, Lewis J, Morgan D, Raff M, Roberts K, Walter P. Stem Cells and Tissue Renewal. *Molecular Biology of the Cell. 6th ed.* 2015:1217-62.
4. Kuderer NM, Dale DC, Crawford J, Lyman GH. Impact of primary prophylaxis with granulocyte colony-stimulating factor on febrile neutropenia and mortality in adult cancer patients receiving chemotherapy: A systematic review. *J Clin Oncol.* 2007;25(21):3158–67.
5. Smith TJ, Khatcheressian J, Lyman GH, Ozer H, Armitage JO, Balducci L, et al. 2006 Update of recommendations for the use of white blood cell growth factors: An evidence-based clinical practice guideline. *J Clin Oncol.* 2006;24(19):3187–205.
6. Bennett CL, Luminari S, Nissenson AR, Tallman MS, Klinge SA, McWilliams N, et al. Pure Red-Cell Aplasia and Epoetin Therapy. *N Engl J Med.* 2004;351(14):1403–8.
7. Zhang Y, Rowley JD. Leukemias, Lymphomas, and Other Related Disorders. *Emery and Rimoin's Principles and Practice of Medical Genetics.* 2013:1–44.
8. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood.* 2016;127(20):2391–405.
9. International Myeloma Working Group. Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders. *Br J Haematol.* 2003;121(5):749–57.
10. Bibas M, Antinori A. EBV and HIV-Related Lymphoma. *Mediterr J Hematol Infect Dis.* 2009;1(2):e2009032.
11. Fitzmaurice C, Akinyemiju TF, Al Lami FH, Alam T, Alizadeh-Navaei R, Allen C, et al. Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-Years for 29 Cancer Groups, 1990 to 2016: A Systematic Analysis for the Global Burden of Disease Study. *JAMA Oncol.* 2018;4(11):1553–68.

12. Sant M, Allemani C, Tereanu C. Incidence of hematologic malignancies in Europe by morphologic subtype: results of the HAEMACARE project. *Blood.* 2011;117(12):3724–34.
13. Pollyea DA, George TI, Abedi M, Bejar R, Cogle CR, Foucar K, et al. Diagnostic and molecular testing patterns in patients with newly diagnosed acute myeloid leukemia in the Connect ® MDS/AML Disease Registry. *eJHaem.* 2020;1:58-68.
14. Hwang SM. Classification of acute myeloid leukemia. *Blood Res.* 2020;55(S1):S1-S4.
15. Blackburn LM, Bender S, Brown S. Acute Leukemia: Diagnosis and Treatment. *Seminars in Oncology Nursing.* 2019;35(6):150950.
16. Türk Hematoloji Derneği. *Multipl Myelom Tanı ve Tedavi Kılavuzu.* 2020:18–56.
17. Wang M, Fayad L, Wagner-Bartak N, Zhang L, Hagemeister F, Neelapu SS, et al. Lenalidomide in combination with rituximab for patients with relapsed or refractory mantle-cell lymphoma: A phase 1/2 clinical trial. *Lancet Oncol.* 2012;13(7):716–23.
18. Barnes DWH, Corp MJ, Louth JF, Neal FE. Treatment of Murine Leukaemia with X Rays and Homologous Bone Marrow. *Br Med J.* 1956;2(4993):626–7.
19. Magenau J, Couriel DR. Hematopoietic Stem Cell Transplantation for Acute Myeloid Leukemia: To Whom, When, and How. *Curr Oncol Reports.* 2013;15(5):436–44.
20. Saadeh S, Litzow M. Hematopoietic stem cell transplant in adults with acute lymphoblastic leukemia: the present state. *Expert Rev Hematol.* 2018;11(3):195–207.
21. Witte T de, Bowen D, Robin M, Malcovati L, Niederwieser D, Yakoub-Agha I, et al. Allogeneic hematopoietic stem cell transplantation for MDS and CMML: recommendations from an international expert panel. *Blood.* 2017;129(13):1753–62.
22. Champlin R, Jabbour E, Kebriaei P, Anderlini P, Andersson B, Lima M de. Allogeneic Stem Cell Transplantation for CML Resistant to Tyrosine Kinase Inhibitors. *Clin Lymphoma Myeloma Leuk.* 2011;11(S1):S96–S100.
23. Gupta A, Khattry N. Current status of hematopoietic stem cell transplant in chronic myeloid leukemia. *Indian J Med Paediatr Oncol.* 2014;35(3):207–10.
24. Gladstone DE, Fuchs E. Hematopoietic stem cell transplantation for chronic lymphocytic leukemia. *Curr Opin Oncol.* 2012;24(2):176–81.
25. Koh LP, Koh MBC, Ng HY, Hwang WYK, Goh YT, Linn YC, et al. Allogeneic Hematopoietic Stem Cell Transplantation for Patients with Severe Aplastic Anemia Following Nonmyeloablative Conditioning Using 200-cGy Total Body Irradiation

and Fludarabine. *Biol Blood Marrow Transplant.* 2006;12(8):887–90.

26. Spivak JL. Myeloproliferative Neoplasms. *N Engl J Med.* 2017;376(22):2168–81.
27. Gaziev J, Lucarelli G. Stem cell transplantation for hemoglobinopathies. *Curr Opin Pediatr.* 2003;15(1):24–31.
28. Zahid U, Akbar F, Amaraneni A, Husnain M, Chan O, Riaz I Bin, et al. A review of autologous stem cell transplantation in Lymphoma. *Curr Hematol Malig Rep.* 2017;12(3):217–26.
29. Morris E, Mackinnon S. Reduced intensity allogeneic stem cell transplantation for low grade non-Hodgkin's lymphoma. *Best Pract Res Clin Haematol.* 2005;18(1):129–42.
30. Rajkumar SV, Kumar S. Multiple Myeloma: Diagnosis and Treatment. *Mayo Clin Proc.* 2016;91(1):101–19.
31. Mora J, Castañeda A, Flores MA, Santa-María V, Garraus M, Gorostegui M, et al. The Role of Autologous Stem-Cell Transplantation in High-Risk Neuroblastoma Consolidated by anti-GD2 Immunotherapy. Results of Two Consecutive Studies. *Front Pharmacol.* 2020;11:575009.
32. Koo JY, Mulcahy-Levy J, Foreman NK, Keating AK, Nuechterlein B, Silverman S. Safety and Feasibility of Outpatient Autologous Hematopoietic Stem Cell Transplantation in Pediatric Patients with Central Nervous System Tumors. *Blood.* 2019;54(10):1605–13.
33. Galgano L, Hutt D. HSCT: How Does It Work? E*ur Blood Marrow Transplant Textb Nurses.* 2017;23–36.
34. Wingard JR, Wirlk B, Brown R. Hematopoietic Stem Cell Transplantation: An overview. *Apheresis: Principles and Practice.* 2010:465–81.
35. Wingard JR, Hsu J, Hiemenz JW. Hematopoietic stem cell transplantation: An overview of infection risks and epidemiology. *Infect Dis Clin North Am.* 2010;24(2):557–72.
36. Luznik L, O'Donnell P V., Symons HJ, Chen AR, Leffell MS, Zahurak M, et al. HLA-Haploidentical Bone Marrow Transplantation for Hematologic Malignancies Using Nonmyeloablative Conditioning and High-Dose, Posttransplantation Cyclophosphamide. *Biol Blood Marrow Transplant.* 2008;14(6):641–50.
37. Klein J, Sato A. The HLA System (First of Two Parts). *N Engl J Med.* 2000;343(10):702–9.

38. Lee SJ, Klein J, Haagenson M, Baxter-Lowe LA, Confer DL, Eapen M, et al. High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation. *Blood.* 2007;110:4576–83.
39. Weisdorf D, Spellman S, Haagenson M, Horowitz M, Lee S, Anasetti C, et al. Classification of HLA-Matching for Retrospective Analysis of Unrelated Donor Transplantation: Revised Definitions to Predict Survival. *Biol Blood Marrow Transplant.* 2008;14:748–58.
40. Ayuk F, Balduzzi A. Donor Selection for Adults and Pediatrics. *The EBMT Handbook.* 2019:87–97.
41. Appelbaum FR, Forman SJ, Blume KG, Negrin RS. *Thomas' Hematopoietic Cell Transplantation. 4th ed.* West Sussex: Wiley-Blackwell; 2009.
42. Mohty M, Harousseau JL. Treatment of autologous stem cell transplant-eligible multiple myeloma patients: Ten questions and answers. *Haematologica.* 2014;99(3):408–16.
43. Özsan H. Otolog Kök Hücre Transplantasyonu'nun Hematolojik Malignansilerin Tedavisindeki Yeri. *HematoLog.* 2011:217–28.
44. L Schwartzberg, R Birch, F Wittlin, J Muscato, K Tauer, B Hazelton, et al. Rapid and sustained hematopoietic reconstitution by peripheral blood stem cell infusion alone following high-dose chemotherapy. *Bone Marrow Transpl.* 1993;11(5):369–74.
45. Lemos NE, Farias MG, Kubaski F, Scotti L, Onsten TGH, Brondani L de A, et al. Quantification of peripheral blood CD34+ cells prior to stem cell harvesting by leukapheresis: a single center experience. *Hematol Transfus Cell Ther.* 2018;40(3):213–8.
46. Ostronoff M, Ostronoff F, Souto Maior P, Matias C, Calixto R, Sucupira A, et al. Pilot Study of Allogeneic G-CSF-Stimulated Bone Marrow Transplantation: Harvest, Engraftment, and Graft-versus-Host Disease. *Biol Blood Marrow Transplant.* 2006;12(7):729–33.
47. Lowenthal RM, Ragg SJ, Anderson J, Nicholson L, Harrup RA, Tuck D. A randomized controlled clinical trial to determine the optimum duration of G-CSF priming prior to BM stem cell harvesting. *Cytotherapy.* 2007;9(2):158–64.
48. Demirer T, Dean Buckner C, Bensinger WI. Optimization of peripheral blood stem cell mobilization. *Stem Cells.* 1996;14(1):106–16.
49. Ford CD, Greenwood J, Anderson J, Snow G, Petersen FB. CD34+ cell adhesion

molecule profiles differ between patients mobilized with granulocyte-colony-stimulating factor alone and chemotherapy followed by granulocyte-colony-stimulating factor. *Transfusion.* 2006;46(2):193–8.

50. Gazitt Y. Comparison between granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor in the mobilization of peripheral blood stem cells. *Current Opinion in Hematology.* 2002;9(3):190–8.
51. Bashey A, Corringham S, Gilpin E, Fields KK, Smilee RC, DeFrancisco C, et al. Simultaneous administration of G-CSF and GM-CSF for re-mobilization in patients with inadequate initial progenitor cell collections for autologous transplantation. *Cytotherapy.* 2000;2(3):195–200.
52. Schwartz J, Padmanabhan A, Francis RO, Linenberger ML. Mobilization and Collection of Peripheral Blood Hematopoietic Stem Cells. *Apheresis: Principles and Practice.* 2010:483–522.
53. Castillo B, Dasgupta A, Klein K, Tint H, Wahed A. Apheresis. *Transfusion Medicine for Pathologists.* 2018:113–24.
54. Abel JJ, Rowntree LG, Turner BB. Plasma Removal With Return of Corpuscles (Plasmapheresis). *J Pharmacol Exp Ther.* 1914;5(6):166–77.
55. Worel N, Mansouri Taleghani B, Strasser E. Recommendations for Therapeutic Apheresis by the Section "Preparative and Therapeutic Hemapheresis" of the German Society for Transfusion Medicine and Immunohematology. *Transfusion Medicine and Hemotherapy.* 2019;46:394–406.
56. Hester JP, Kellogg RM, Mulzet A, McCredie KB, Freireich EJ. Principles of Continuous Flow Cell Separation in a Circumferential Flow Disposable Channel. *Methods of Cell Separation.* 1980:101–13.
57. Hester JP, Kellogg RM, Mulzet AP, Freireich EJ. Continuous-flow techniques for platelet concentrate collection: A step toward standardization and yield predictability. *J Clin Apher.* 1985;2(3):224–30.
58. Schwartz J, Winters JL, Padmanabhan A, Balogun RA, Delaney M, Linenberger ML, et al. Guidelines on the Use of Therapeutic Apheresis in Clinical Practice—Evidence-Based Approach from the Writing Committee of the American Society for Apheresis: The Sixth Special Issue. *J Clin Apher.* 2013;28(3):145–284.
59. Torey CA, Snyder EL. Hematopoietic progenitor cell administration. *Hematopoietic stem cell transplantation: A handbook for clinicians.* 2009:151–62.

60. Mohammadi S, Mohammadi AM, Nikbakht M, Norooznezhad AH, Alimoghaddam K, Ghavamzadeh A. Optimizing stem cells mobilization strategies to ameliorate patient outcomes: A review of guide lines and recommendations. *International Journal of Hematology-Oncology and Stem Cell Research.* 2017;11(1):78–88.
61. Bender JG, To LB, Williams S, Schwartzberg LS. Defining a Therapeutic Dose of Peripheral Blood Stem Cells. *J Hematotherapy Stem Cell Res.* 1992;1(4):329–41.
62. Burgstaler EA, Pineda AA. Therapeutic plasma exchange: A paired comparison of Fresenius AS104 vs. COBE Spectra. *J Clin Apher.* 2001;16(2):61–6.
63. Hester JP, Ventura GJ, Boucher T. Platelet concentrate collection in a dual-stage channel using computer-generated algorithms for collection and prediction of yield. *Plasma Ther Transfus Technol.* 1987;8(4):377–85.
64. Hester JP, Ventura J. Mathematic Modeling for Apheresis Procedures; Advantages and Limitations. *Automation in blood transfusion.* 1989:121–7.
65. Richa E, Krueger P, Burgstaler EA, Bryant SC, Winters JL. The effect of double- And triple-apheresis platelet product donation on apheresis donor platelet and white blood cell counts. *Transfusion.* 2008;48(7):1325–32.
66. AbuSamra DB, Aleisa FA, Al-Amoodi AS, Ahmed HMJ, Chin CJ, Abuelela AF, et al. Not just a marker: CD34 on human hematopoietic stem/progenitor cells dominates vascular selectin binding along with CD44. *Blood Adv.* 2017;1(27):2799–816.
67. Lane TA. Peripheral blood progenitor cell mobilization and collection. *Hematopoietic stem cell teraphy.* 2000:269–86.
68. Law P. Graft, processing, storage, and infusion. *Hematopoietic stem cell teraphy.* 2000:312–21.
69. Shi Y, Liu CH, Roberts AI, Das J, Xu G, Ren G, et al. Granulocyte-macrophage colony-stimulating factor (GM-CSF) and T-cell responses: What we do and don't know. *Cell Res.* 2006;16(2):126–33.
70. Al-Shami A, Mahanna W, Naccache PH. Granulocyte-Macrophage Colony-stimulating Factor-activated Signaling Pathways in Human Neutrophils. *J Biol Chem.* 1998;273(2):1058–63.
71. Lanza F, Castagnari B, Rigolin G, Moretti S, Latorraca A, Ferrari L, et al. Flow cytometry measurement of GM-CSF receptors in acute leukemic blasts, and normal hemopoietic cells. *Leukemia.* 1997;11(10):1700–10.
72. Thomas GD, Hamers AAJ, Nakao C, Marcovecchio P, Taylor AM, McSkimming C,

et al. Human Blood Monocyte Subsets. *Arterioscler Thromb Vasc Biol.* 2017;37(8):1548–58.

73. Lee J, Tam H, Adler L, Ilstad-Minnihan A, Macaubas C, Mellins E. The MHC class II antigen presentation pathway in human monocytes differs by subset and is regulated by cytokines. *PLoS One.* 2017;12(8):e0183594.
74. Kimura F. Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF). *Nippon Rinsho.* 2003;57:202–8.
75. Sutherland DR, Anderson L, Keeney M, Nayar R, Chin-Yee I. The ISHAGE Guidelines for CD34+ Cell Determination by Flow Cytometry. *J Hematother.* 1996;5(3):213–26.
76. Almeida-Neto C, Rocha V, Moreira F, Hamasaki D, Farias M, Arrifano A, et al. Validation of a formula predictive of peripheral blood stem cell yield and successful collection in healthy allogeneic donors. *Hematol Transfus cell Ther.* 2020;42(2):164-165.e5.
77. Bethge W, Federmann B, Bornhauser M, Meisner C, Kordelas L, Beelen DW, et al. Stem Cell Transplantation Haploidentical allogeneic hematopoietic cell transplantation in adults using CD3/CD19 depletion and reduced intensity conditioning: a phase II study. *Haematologica.* 2012;97(10):1523–31.
78. Mizrahi O, Ish Shalom E, Baniyash M, Klieger Y. Quantitative Flow Cytometry: Concerns and Recommendations in Clinic and Research. *Cytom Part B Clin Cytom.* 2018;94(2):211–8.
79. Minimum information about a flow cytometry experiment (MIFlowCyt) checklist (Numbered in accordance with MIFlowCyt 1.0 document). *Cytometry A.* 2010;77(9):813.
80. Ferrand VL, Montero Julian FA, Chauvet MM, Hirn MH, Bourdeaux MJ. Quantitative Determination of the MDR-Related P-Glycoprotein, Pgp 170, by a Rapid Flow Cytometric Technique. *Cytometry.* 1996;23(2):120–5.
81. Sirinoglu Demiriz I, Tekgunduz E, Altuntas F. What Is the Most Appropriate Source for Hematopoietic Stem Cell Transplantation? Peripheral Stem Cell/Bone Marrow/Cord Blood. *Bone Marrow Res.* 2012;2012:834040.
82. Cheuk DK. Optimal stem cell source for allogeneic stem cell transplantation for hematological malignancies. *World J Transplant.* 2013;3(4):99–112.
83. Nikolajeva O, Mijovic A, Hess D, Tatam E, Amrolia P, Chiesa R, et al. Single-donor

granulocyte transfusions for improving the outcome of high-risk pediatric patients with known bacterial and fungal infections undergoing stem cell transplantation: A 10-year single-center experience. *Bone Marrow Transplant.* 2015;50(6):846–9.

84. Griffin JD, Spertini O, Ernst TJ, Belvin MP, Levine HB, Kanakura Y, et al. Granulocyte-macrophage colony-stimulating factor and other cytokines regulate surface expression of the leukocyte adhesion molecule-1 on human neutrophils, monocytes, and their precursors. *J Immunol.* 1990;145(2):576–84.
85. Islam A. Pattern of bone marrow regeneration following chemotherapy for acute myeloid leukemia. *J Med.* 1987;18(2):108–22.
86. Corso A, Caberlon S, Pagnucco G, Klersy C, Zappasodi P, Alessandrino EP, et al. Blood stem cell collections in multiple myeloma: Definition of a scoring system. *Bone Marrow Transplant.* 2000;26(3):283–6.
87. Tiwari AK, Pandey P, Subbaraman H, Bhargava R, Rawat G, Madiraju S, et al. Autologous peripheral blood stem cell harvest: Collection efficiency and factors affecting it. *Asian J Transfus Sci.* 2016;10(1):93–7.

APPENDIX A: ETHICAL COMMITTEE APPROVAL FORM

T.C. YEDİTEPE ÜNİVERSİTESİ

Sayı : 37068608-6100-15- 1831 **05/03/2020**
Konu: Klinik Araştırmalar
Etik kurul Başvurusu hk.

İlgili Makama (Ayşe Yiğit)

Yeditepe Üniversitesi Tıp Fakültesi İmmünoloji ABD Prof. Dr. Gülderen Yanıkkaya Demirel'in sorumlu araştırmacı olduğu **"Aferez İle Toplanan Periferik Kök Hücre Örneklerinde Farklı Hücre Grupları Üzerinde G-CSF Reseptör ve GM-CSF Reseptör Sayılarının Karşılaştırılması İle Otolog Transplantasyon İçin Prediktif Faktör Olarak Değerinin Belirlenmesi''** isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KAEK) Başvuru Dosyası **(1816)** kayıt Numaralı KAEK Başvuru Dosyası, Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından **04.03.2020** tarihli toplantıda incelenmiştir.

Kurul tarafından yapılan inceleme sonucu, yukarıdaki isimi belirtilen çalışmanın yapılmasının etik ve bilimsel açıdan uygun olduğuna karar verilmiştir (**KAEK Karar No: 1172**).

Prof. Dr. Turgay ÇELİK
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