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Effect of Mobilization Methods on CD34+ and Total Nucleated Cell Count and Their relation with Engraftment in Autologous Hematopoietic Stem Cell Transplantation

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ABSTRACT

Effective stem cell mobilization is crucial for autologous hematopoietic stem cell transplantation (auto-HSCT), impacting engraftment time and patient outcomes. This study compared three mobilization regimens (G-CSF alone, G-CSF & plerixafor, and chemotherapy & G-CSF) to determine their optimal use for maximizing CD34+ cell yield and minimizing engraftment times.

A retrospective analysis of 227 auto-HSCT patients was conducted. Mobilization groups, CD34+ cell counts, total nucleated cells (TNC), total mononuclear cells (TMNC), and platelet/neutrophil engraftment times were analyzed.

The average engraftment time was 11.6 days for platelets and 10.6 days for neutrophils. The chemotherapy & G-CSF arm yielded the highest CD34+ cells (p=0.001) and lowest TNC/TMNC (p=0.000). This arm also achieved the fastest platelet engraftment (p=0.017-0.001). Notably, age positively correlated with TNC count (p=0.022) and prolonged neutrophil engraftment (p=0.021). Gender did not significantly influence engraftment times.

The chemotherapy & G-CSF regimen yielded the highest CD34+ cell count, lowest TNC/TMNC, and fastest platelet engraftment. However, neutrophil engraftment was positively associated with age, suggesting additional factors influence this outcome. Optimizing mobilization strategies and considering patient age are crucial for optimizing auto-HSCT outcomes.

Keywords: Stem cell, engraftment, CD34

Introduction

Hematopoietic cell transplantation is potentially curative treatment option for malignant and non-malignant diseases. (1) The multipotent hematopoietic stem cells required for this procedure are obtained from the donor's bone marrow or peripheral blood (PB). (2) PB is the most common source for autologous hematopoietic stem cell transplantation (auto-HSCT). (3) According to the knowledge; three ways are used for stem cell collection from PB, using only granulocyte colony stimulating factor (G-CSF), G-CSF and plerixafor, chemotherapy and G-CSF.[3] The biggest problem after Auto-HSCT is the engraftment time of platelets and neutrophils. Engraftment should be rapid after Auto-HSCT (5), therefore the quality and quantity of CD34+ cells is important. (4) Ideal products include high levels of CD34+ cells with low levels of total nucleated cells (TNC) and total mononucleotide cells (TMNC). (3) Although avoiding TNC and TMNC, in addition to CD34+ cell, TNC and TMNC are infused to the patient as they are in the product obtained during stem cell collection.(5) The quantity and quality of stem cells are determined by complications such as length of hospital stay, risk of bleeding or infection due to cytopenias, insufficiency or failure of engraftment. (4,6)

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Our aim is to investigate whether the number of $CD34^+$ cells and TNC and TMNC varies according to the methods of PB stem cell mobilization in auto-HSCT and how the content of the product obtained affects the engraftment time of platelets and neutrophils.

Material and Methods

All patients who underwent auto-HSCT between 2012-2020 are included. Donors for allogeneic stem cell transplantation are excluded. The patients did not have CMV infection or use of bone marrow suppressive antibiotics. Retrospective analysis of 227 auto-HSCT patients' records were included. Patient information is shown in Table 1. Mobilization regimen were categorized into three groups; mobilized with G-CSF only (first group), G-CSF & plerixafor (second group) and chemotherapy and G-CSF (third group). For the G-CSF group; 10 mcg/kg G-CSF was administered for four days. On the fourth day, stem cells were collected. For the second group: the recommended protocol for mobilization included a daily injection of G-CSF (10 mcg/kg) administered subcutaneously (s.c.) for four consecutive days before plerixafor was administered. On the evening of the fourth day, patients received an injection of plerixafor (0.24 mg/kg s.c.) 10-12 hours prior to the scheduled stem cell collection. The next morning, patients were advised to receive their G-CSF injection at least one hour before apheresis. This procedure was repeated until either the desired number of CD34+ cells was collected or a failure of mobilization was diagnosed. In the third group, cyclophosphamide was administered at 3000 mg/m^2 for one day, followed by G-CSF at 10 microg/kg sc on the second day of cyclophosphamide. Ten days after cyclophosphamide therapy, stem cell collection was planned if the absolute neutrophil count was $>10x10^9/L$. In the latter case, the investigators could decide to discontinue the mobilization procedure if the patient did not release a satisfactory number of CD34+ cells into the PB or if they did not collect enough cells to warrant continuing. Numbers of nucleated cells and CD34+ cells in PB and apheresis products were determined in local laboratories. Neutrophil engraftment was defined as the first day after a neutrophil nadir of 3 consecutive measurements with an absolute neutrophil count of at least $0.5\times10^9/L$. Platelet engraftment was defined as the first day after nadir with a rising platelet count of at least $20\times10^9/L$ maintained without transfusion support.

Statistical Methods: Descriptive statistics were used to summarize the demographic and clinical characteristics of the patients. Categorical variables such as patients' age, gender, and diagnosis were presented as frequency and percentage values. Numerical variables such as CD34+, TNC, TMNC, platelet, and neutrophil engraftment times were presented as mean, standard deviation, minimum, and maximum values. Two-sample t-tests were used to compare CD34+, TNC, TMNC, platelet, and neutrophil engraftment times between the groups. Independent t-tests were used. The normality assumption was checked with the [normality test] test. A p-value of < 0.05 was considered statistically significant. All analyses were carried out using 'SPSS Statistics V.26' SPSS Inc, Chicago, IL, USA statistical software.

Results

The study population comprised patients with an average age of 49 years (range: 18-71 years). Mean platelet engraftment time was 11.6 days, while mean neutrophil engraftment time was 10.6 days. In comparison with the age variable, it was found to be statistically significant that there was an increase in the number of TNCs as age increased ($p = 0.018$). A significant relationship was found between age and neutrophil engraftment time; as age increased, neutrophil engraftment time increased ($p=0.021$). When the number of CD34+ positive cells was analyzed among the three groups, a significant difference was observed in the chemotherapy and GCSF arm compared to the GCSF and G-CSF & plerixafor arm ($p=0.001$). The number of TNC and TMNC was least determined in the chemotherapy and GCSF arm compared to the other two groups ($p = 0.001$). There was no statistically significant difference between the use of G-CSF alone and G-CSF plus plerixafor in terms of platelet engraftment times ($p = 0.254$). There was a statistically significant difference in platelet engraftment time when the chemotherapy and GCSF arm were compared with the other two groups ($p = 0.017$ for G CSF alone and $p = 0.001$ for plerixafor with G CSF). A significant relationship was found between increased CD34+ cell count and platelet engraftment ($p = 0.01$). No significant relationship was detected between increased CD34+ cell count and neutrophil engraftment time ($p = 0.875$). There was no statistically

Table 1: Baseline clinical and vemographic characteristics of patients

Parametres	
Median age at HSCT (range), years	54 (18-71)
Male/female [n/n]	148/79
Type of disease	
Multiple myeloma [n(%)]	129(56.8%)
Hodgkin lymphoma [n(%)]	30(13.2%)
Non-hodgkin lymphoma [n(%)]	51(22.5%)
Ewing sarcoma [n(%)]	1(0.4%)
Medulloblastoma [n(%)]	1(0.4%)
Testicular cancer [n(%)]	13(5.7%)
Waldenström macroglobulinemia [n(%)]	1(0.4%)
Mobilization methods	
G-CSF [n(%)]	30 (13.2)
G-CSF & plerixafor [n(%)]	35 (15.4)
Chemotherapy + filgrastim:	162 (7.14):
(DHAP + filgrastim)	5
(ICE +filgrastim)	7
(Cyclophosphamide + filgrastim)	150

Table 2: CD34+ cell, TNC, TNMC count and engraftment times according to the mobilization methods

Mobilization methods	CD 34+ cell count 106/kg (mean)	TNC count 106/kg (mean)	TMNC count 106/kg (mean)	Platelet engraftment days (mean)	Neutrophil engraftment days (mean)
G-CSF	4.2133	16.7233	6.8840	12.17	10.77
G-CSF & plerixafor	3.5083	13.9343	6.8943	15.37	11.74
G-CSF & chemotherapy	7.3048	8.0219	3.5383	10.70	10.43

significant difference in platelet engraftment time (p=0.084) and neutrophil engraftment time (p=0.041) according to gender variable. Table-2 shows the amounts of CD34+ cells, TNC, TMNC, platelet and neutrophil engraftment times according to the three mobilization groups.

Discussion

In autologous hematopoietic stem cell transplantation (Auto-HSCT), the quantity and quality of the infused product after high-dose chemotherapy are crucial. Our study investigates the most suitable stem cell mobilization method in terms of engraftment time, which can lead to early discharge and a decrease in complications related to cytopenia, such as bleeding and infection.

The relationship between the number of nucleated cells and donor characteristics has been studied in several research studies.(7,8) In patients who underwent allogeneic stem cell transplantation, those who received higher numbers of TNCs had better PFS.9 In pediatric patients who underwent allogeneic stem cell transplantation, TNC dose was found to be a better prognostic factor than doses of CD34+ cells, CD3+ cells, or TMC.10 In another retrospective study, no significant difference was observed between the number of nucleated cells and the engraftment times.11 Similarly, a review of peripheral Auto-HSCT data in pediatric patients from the literature indicates that there is no correlation between engraftment and the dose of TNC cells per kg or CD34+ cells per kg.12

In our study, the mean engraftment time was 11,6 days for platelets and 10,6 days for neutrophils, with the longest engraftment time being 55 days for platelets and 51 days for neutrophils. The chemotherapy and G-CSF arm showed a significantly higher number of CD34+ positive cells compared to the other two groups.

Additionally, this arm had the least number of TNC and TMNC compared to the G-CSF and G-CSF & plerixafor arm. This group was observed to be the most efficient in terms of product quality, and platelet engraftment was faster in the chemotherapy and G-CSF arm. It is suggested that the observed difference may be attributed to the higher number of CD34+ cells and lower number of nucleated cells in the product obtained from the chemotherapy and G-CSF arm. While high doses of CD34+ cells are linked to faster platelet engraftment, neutrophil engraftment is not affected. Neutrophil engraftment can be affected by several factors, such as the dose of CD34+ cells, disease stage, and pretransplant radiotherapy. The dose of CD34+ cells was found to be an independent factor for platelet engraftment. (13,14) Additionally, this issue can be explained by another finding in this study: there was a positive correlation between uncategorized age and the number of TNCs, as well as the duration of neutrophil engraftment. It was discovered that the number of TNCs in the collected product increased significantly, particularly in the group of individuals over 50 years old. A positive correlation was found between TNC count and age above 50. This, in turn, affects the quality of the obtained product and prolongs the neutrophil engraftment time.

The mobilization group that received chemotherapy and G-CSF showed the highest CD34+ cell count, the lowest TNC count, and the fastest platelet engraftment in our study. We also observed a prolonged neutrophil engraftment and an increase in the number of TNCs in patients over 50 years of age. In addition to obtaining CD34+ positive cells, product quality, number of TNCs, and age are factors that affect engraftment time.

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