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Stem Cell Mobilization Efficiency and Engraftment Kinetics in Patients with Hematologic Malignancies Undergoing Autologous Stem Cell Transplantation: A Retrospective Cohort Study*

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ABSTRACT

Objective: This study aimed to conduct a retrospective evaluation of clinical findings on patients undergoing autologous hematopoietic stem cell transplantation.

Materials and Methods: A total of 167 consecutive patients who were diagnosed with multiple myeloma (MM) and lymphoma and then underwent autologous hematopoietic stem cell transplantation (AHSCT) between August 2010 and May 2013 were included in our study. Demographic, disease, mobilization, apheresis, and transplantation data were reviewed from patient files.

Results: In 121 patients (72%), mobilization was achieved solely with granulocyte colony stimulating factor (G-CSF). There was no relationship between peripheral $CD34^+$ cell count and age, disease type, or previous treatment features. The total $CD34^+$ cell count post-apheresis was $3.3 \pm 3.1 \times 10^6$/kg. Only nine patients could not achieve successful mobilization with any regimen. The median day of neutrophil and platelet engraftment among the entire patient group was 11 days. As the number of $CD34^+$ cells infused into patients increased, neutrophil and platelet engraftment time decreased.

Conclusion: Mobilization was achieved in most MM cases and at least two-thirds of lymphomas using G-CSF alone. Age and body weight did not affect mobilization success. Clinicians should increase successful mobilizations on the first day of the apheresis and prescribe AHSCT at appropriate times to avoid excessive cycles of chemotherapy.

Keywords: Mobilization, apheresis, autologous stem cell transplantation, high-dose chemotherapy

INTRODUCTION

Multiple myeloma (MM) is the malignant proliferation of plasma cells originating from a single clone. It may manifest as a range of organ dysfunctions, bone pain, fractures, kidney failure, susceptibility to infections, anemia, hypercalcemia, coagulation abnormalities, neurological symptoms, and signs of hyperviscosity (1). In these cancers, immune cells differentiate at various stages, leading to a broad spectrum in morphological, immunological, and clinical presentations that range from indolent to aggressive. Cancers of the lymphatic system often present as leukemia, which primarily involves bone marrow and peripheral blood, whereas others are lymphoma, solid tumors of the immune system (2). In MM, autologous hematopoietic stem cell transplantation (AHSCT) supported by high-dose chemotherapy remains

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the standard treatment for patients under 65 years old and those from 65–75 years old who respond well to induction therapy (3). Previous studies have suggested that AHSCT followed by high-dose chemotherapy or immunotherapy is an appropriate treatment option for relapsed cases of chemotherapy-sensitive patients with diffuse large B-cell lymphomas as well as refractory/relapsed Hodgkin patients (4, 5).

Hematopoietic stem cells (HSCs) are a unique subset of cells within the hematopoietic system that can differentiate into any blood cell type while maintaining their capacity for self-renewal. Studies revealed increased numbers of HSCs in peripheral blood during post-chemotherapy recovery, suggesting their role in hematopoietic regeneration. It was found in the 1980s that peripheral HSCs contribute significantly to full hematopoietic recovery following myeloablative therapy, informing future stem cell studies.

There are three different types of hematopoietic stem cell transplantations (HSCTs): Allogeneic HSCT uses healthy blood stem cells from a donor or cord blood stem cell source. Syngeneic HSCT relies on blood stem cells from an identical twin, where both the donor and recipient are monozygotic. Autologous HSCT, on the other hand, collects HSCs from a patient that are subsequently re-transplanted into the same individual.

Administration of granulocyte colony-stimulating factor (G-CSF) or granulocyte–macrophage colony stimulating factor (GM-CSF) alone increases the number of HSCs in peripheral blood, a process called stem cell mobilization; chemomobilization relies on chemotherapy/chemotherapy followed by cytokine administration (G-CSF) or G-CSF in combination with plerixafor, a selective C-X-C chemokine receptor type 4 (CXCR4) antagonist. These agents synergistically elevate the number of circulating HSCs, which can be collected through apheresis (6), a critical step in stem cell transplantation in which blood components are separated and desired cells are collected. Equipment and methods such as filtration and centrifugation have been developed to optimize these steps and obtain purer stem cell products. For example, filtration utilizes differences in cell density to isolate and separate blood components (7). Ultimately, the success of AHSCT-supported high-dose chemotherapy depends on the quantity and viability of cells collected through apheresis. Generally, at least 2×10^6 $CD34^+$ cells/kg must be collected for complete and rapid hematopoietic recovery. Moreover, a product with $>5 \times 10^6$ $CD34^+$ cells/kg accelerates neutrophil and platelet engraftment while reducing hospitalization and costs (8).

Retrospective studies can show how patients respond to treatment and how disease progresses over time. The current study aims to fulfill several objectives in the Hematology Unit's apheresis facility: conduct a retrospective evaluation of patients undergoing treatment for Hodgkin's lymphoma (HL), non-Hodgkin's lymphoma (NHL), and MM; assess the accurate and effective applicability of international treatment protocols; optimize patient outcomes; and recommend ways to improve standard treatments .

MATERIALS AND METHODS

Study Group

This study was conducted at the Hematopoietic Stem Cell Transplantation Unit of Demiroglu Bilim University, Sisli Florence Nightingale Hospital. The study included patients diagnosed with HL, NHL, or MM between August 2010 and May 2013. This study was approved by the Demiroglu Bilim University Clinical Research Ethics Committee on April 11, 2013 (44140529/2013-036). These patients were either referred to our center for AHSCT or were already undergoing treatment for the same diagnoses at our hospital. Patients over the age of 18 who had an indication for AHSCT were consecutively included in the study. Patients who were diagnosed with a cancer other than lymphomas or MM, underwent HSCT collection at an external center and received AHSCT at our hospital, or were under 18 years old were not included in the study. Patients whose cells were to be cryopreserved after apheresis were included in apheresis kinetic studies but not in engraftment kinetic analyses. Demographics, disease characteristics (stage, chemotherapy, radiotherapy), mobilization (mobilization regimens: G-CSF alone, G-CSF and chemotherapy, G-CSF and plerixafor; total G-CSF dose used, number of days G-CSF was used), apheresis data (catheter) type, device type, peripheral CD34 positive ($pCD34^+$) cell count, apheresis date, total blood volume processed, processed blood volume, anti-coagulation, procedure duration, pre-apheresis leukocyte count, pre-apheresis mononuclear cells count, post-apheresis $CD34^+$ cell count, post-apheresis mononuclear cell count, product volume, total dimethyl sulfoxide (DMSO) content, and transplant data (conditioning regimen, number of infusion days, infused $CD34^+$ cell count, bag count, infused volume, neutrophil and platelet engraftment date) were recorded for each patient. Bedside apheresis procedures were conducted using two different continuous-flow apheresis devices: the Spectra Optia Apheresis System (Caridian BCT Inc., Lakewood CO, USA) and the Fresenius COM.TEC (Fresenius Kabi, Freidberg, Germany). The Spectra Optia Collection Set and the Fresenius P1Y-P1YA sets were utilized through apheresis. Patients were classified based on their body mass index (BMI) as underweight (below 20 kg/m^2), normal weight (20–25.9 kg/m^2), overweight (26–29.9 kg/m^2), or obese (above 30 kg/m^2). Supplementary Table 1 shows the formulas used to calculate BMI and other indices (Ideal Body Weight, IBW; Actual Body Weight, ABW; and Adjusted Ideal Body Weight, AdjIBW).

Quantification and Characterization of $pCD34^+$ Cells

A complete blood count was performed on peripheral blood samples from patients using the Sysmex XT-2000i Automatic Hematology Analyzer, and leukocyte counts were verified using a Thoma cell counting chamber.

To analyze pCD34$^+$ cells, whole blood staining was performed using anti-human phycoerythrin (PE) (BD Biosciences, USA) and anti-human CD45 fluorescein isothiocyanate (FITC) (BD Biosciences, USA) monoclonal antibodies (100 µL of whole blood and 10 µL of each monoclonal antibody). The prepared mixture was incubated in the dark at room temperature for 20 minutes, followed by the addition of 2 mL of FACS™ lysing solution (BD Biosciences, USA) 10x concentrate for another 15-minute incubation in the dark at room temperature. After washing, the cell suspension was analyzed using a flow cytometry device (Beckman Coulter, Epics XL-, System 3) as prescribed by the International Society of Hematotherapy and Graft Engineering protocol for cells not marked with 7-Amino-Actinomycin D (7AAD) (BD Biosciences, USA), indicating they were alive (negative = alive) (9). The flow cytometry gating and analysis strategy was as follows:

- All cells with weak and strong staining for CD45 (positive) (CD45 / SS) were gated (Gate A). Thus, all erythrocytes, platelets, and debris were excluded, and leukocyte gating was defined.
- Gate A for CD34 was introduced in the SS graph of CD34 (CD34 / SS). Cells with low SS and CD34 expression (positive) were gated (Gate B).
- Subsequently, cells from Gates A and B were introduced into the SS graph for CD45. In this graph, cells with low SS and weak to moderate fluorescence for CD45 were gated (Gate C).
- Cells were analyzed based on their size and granularity in the forward scatter (FS)/SS graph. Cells in Gates A+B+C showed low SS and a homogeneous distribution in FS (forward scatter); they were gated (Gate F).
- In the A graph, all leukocytes were gated; lymphocytes stained strongly for CD45 (Gate D). Gate D was introduced into the E graph, and homogeneously distributed lymphocytes in the graph were gated.
- Without gating, cells were evaluated for pCD34$^+$ with weak CD45 fluorescence (CD45/CD34).
- Cells positive for 7AAD (dead) were excluded from the pool of 7AAD-negative (live) cells.

Characterization of Collected CD34$^+$ Cell Quantity

Apheresis was performed at the patient's bedside using two different continuous flow apheresis devices: the Caridian BCT Inc. Spectra Optia Collection Set Apheresis System and the Fresenius P1Y-P1YA Comtec. The product obtained from each patient through apheresis was drawn into a syringe within a sterile cabinet and its volume recorded. The total CD34$^+$ cell count was obtained by multiplying the CD34$^+$ cell count detected per microliter by the product volume. The number of CD34$^+$ cells per kilogram for each patient was determined as follows:

$$\text{CD34+ cells} = \frac{\text{(CD34+ count per microliter) x (product volume in microliters)}}{\text{patient's body weight in kilograms}} \text{ x } 100$$

Storage and Use of CD34$^+$ Cells

Products containing 0.5 × 10^6/kg CD34$^+$ cells were primarily cryopreserved in our center using 6% hydroxyethyl starch (HES) and 7.5% DMSO as a cryoprotective. These products were stored in liquid nitrogen tanks inside CryoStore CS750NS containers in Oni-Gen bags in our facility. Frozen cells were thawed in a water bath at the Apheresis Unit and counted again.

High-Dose Chemotherapy

All patients diagnosed with MM received high-dose melphalan as the conditioning regimen. Day 0 was defined as the infusion day, and on day 2, 200 mg/m^2/day of melphalan (with dose adjustments made based on patient characteristics) were administered intravenously over two hours. Nearly all patients diagnosed with lymphoma underwent carmustine plus etoposide plus cytarabine plus melphalan (BEAM) conditioning, where day 0 was defined as the infusion day. On days 7, 6, 5, 4, and 3, carmustin 300 mg/m^2 (over two hours), etoposide 200 mg/m^2 (over two hours), and cytarabine (over two hours) were administered, and on day 2, melphalan 140 mg/m^2 (over one hour) was administered intravenously. One patient diagnosed with NHL received ifosfamide plus carboplatin plus etoposide (ICE) therapy as the conditioning regimen, where day 0 was the infusion day. Ifosfamide 2.5 g/m^2 (over 24 hours), mesna 2.5 g/m^2 (over 24 hours), and etoposide 300 mg/m^2 (x2, over two hours) on days 6, 5, 4, and 3 as well as carboplatin 500 mg/m^2 (over two hours) on days 6, 5, and 4 were administered intravenously.

Hematopoietic Stem Cell Transplantation

After thawing according to the conditioning regimen protocol on day 0, medications were administered through the central venous catheter in compliance with infusion guidelines. The period of pancytopenia that developed in patients following transplantation was addressed with support therapies, antibiotics based on culture results, and, when necessary, blood product replacements.

Statistical Analyses

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) 21.0 program. Graphs were created using SPSS 21.0 and GraphPad Prism 5. Variance analysis between patient groups was conducted using ANOVA, and Mann–Whitney U test was applied for inter-group comparisons of parameters that were not normally distributed. Spearman correlation analysis was performed to determine the relationship between variables. Nonparametric categorical data were compared with the chi-square test or Fisher's exact

test as necessary. Patient demographic data were presented as the mean with standard deviation; medians were presented in figures. p-values <0.05 were considered significant.

RESULTS

Demographic and Clinical Characteristics

We examined the files of 167 patients who were diagnosed as follows: 31 with HL, 55 with NHL, and 81 with MM. The gender distribution among the patients was 57 (34.2%) female and 110 (65.8%) male. Patients ranged from 18 to 72 years old, with a mean age of 52 ± 13 years. Detailed demographics of the cohort are shown at Table 1. The average IBWs were 67.6 ± 6.9 and 50.6 ± 5.6 kg, in males and females, respectively. Average ABWs were 73.4 ± 8.5 kg and 79.9 ± 7.9 kg, respectively. Lastly, the average AdjIBWs were 69 ± 6.9 kg for males and 52.4 ± 5.4 kg for females.

Prior to the decision for high-dose therapy, four (12.9%) HL cases and nine (16.4%) NHL cases were chemorefractory, whereas 27 (87.1%) HL cases and 46 (83.6%) NHL cases were chemosensitive. Among the MM cases, 17 (20.9%) were in partial remission, 22 (27.2%) were in very good partial remission, 40 (49.5%) were in complete remission, and two (2.4%) had progressive disease when high-dose chemotherapy and AHSCT were decided upon.

Mobilization

Mobilization was achieved solely with G-CSF in 121 (72%) patients. Post-chemotherapy mobilization was performed in six (3.6%) patients. In five (6%) patients, post-chemotherapy mobilization failed initially, but successful mobilization was subsequently achieved with G-CSF. In three patients, including two MM and one NHL patient, bone marrow was collected because current regimens could not achieve mobilization. G-CSF and cyclophosphamide were used as the first-line mobilization regimen, but apheresis was not performed due to insufficient pCD34⁺ cell yield in one patient.

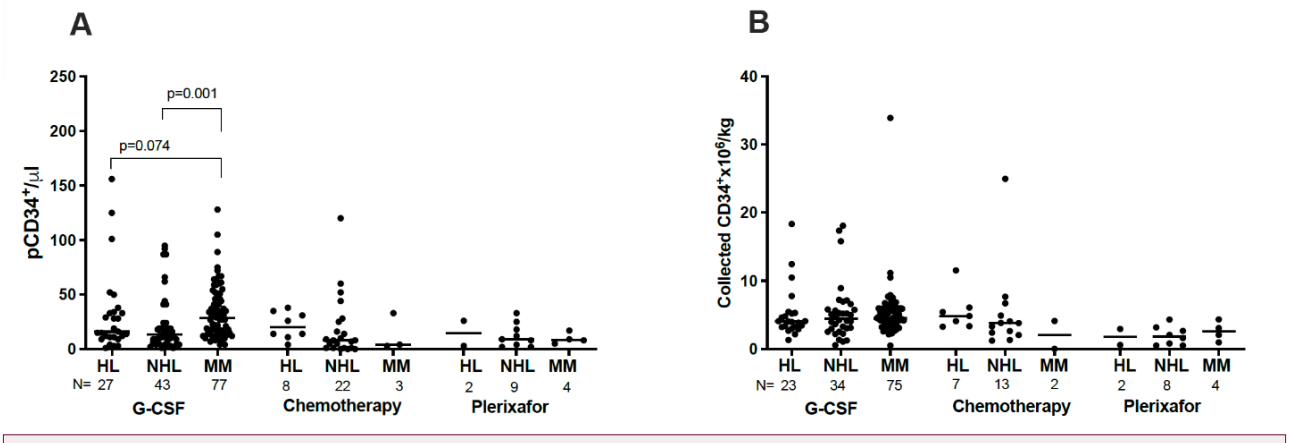

Figure 1. A. pCD34⁺ cell counts for each mobilization regimens and disease; B. Collected CD34⁺ cell counts for each mobilization regimen and diseases. HL: Hodgkin's lymphoma; NHL: Non-Hodgkin's lymphoma; MM: Multiple myeloma.

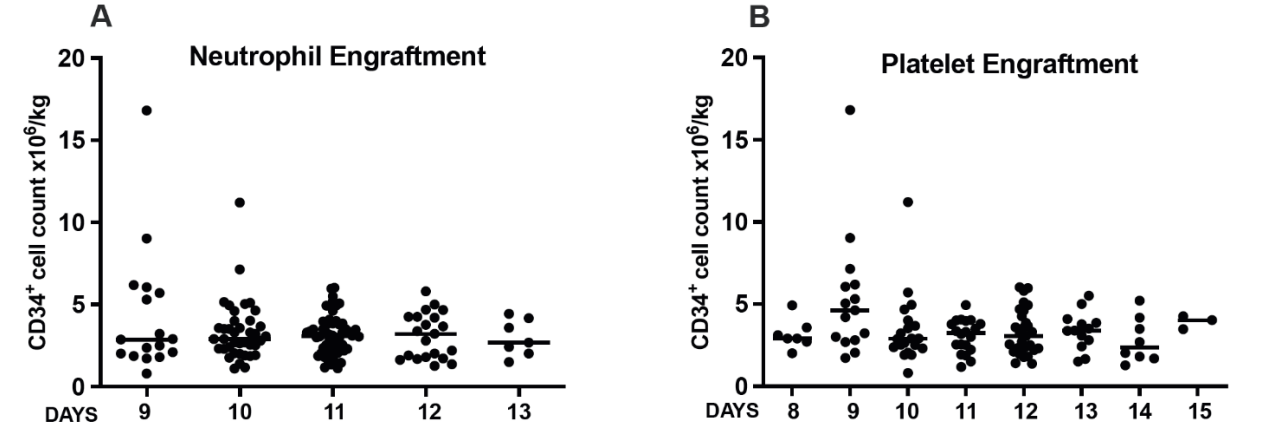

Figure 2. A. The effect of CD34⁺ cell counts on neutrophil engraftment, B. The effect of CD34⁺ cell counts on platelet engraftment.

Table 1. Demographic and clinical characteristics of the cohort.

	HL (n=31)	NHL (n=55)	MM (n=81)
Women (%)	35.5	34.5	33.3
Age, years, mean±SD	37 ± 12.7	47.4 ± 13.9	56.6 ± 7.9
Underweight (%)	6.5	9.1	2.5
Normal weight (%)	48.4	34.5	27.2
Overweight (%)	19.4	29.1	33.3
Obese (%)	25.8	27.3	37
Ideal Body Weight (IBW) kg, mean±SD	62.85 ± 10.7	62.97 ± 11.0	60.58 ± 9.7
Actual Body Weight (ABW) kg, mean±SD	68.23 ± 12.7	68.71 ± 11.6	67.65 ± 10.1
Adjusted Ideal Body Weight (AdjIBW) kg, mean±SD	64.19 ± 10.9	64.40 ± 10.8	62.35 ± 9.5
Radiotherapy (%)	32.3	32.7	43.2

HL: Hodgkin's lymphoma; NHL: Non-Hodgkin's lymphoma; MM: Multiple myeloma; SD: Standard Deviation

Radiotherapy did not significantly affect the yield of $pCD34^+$ cells (35.25 ± 40 mL vs. 29.25 ± 25.7 mL, p = 0.31). The number of collected $CD34^+$ cells after G-CSF administration decreased with an increasing number of chemotherapy cycles received, but this did not reach statistical significance. In patients who received one, two, and three series of chemotherapy, the mean $pCD34^+$ cell count was 25, 23, and 14 /µL, respectively.

The median $pCD34^+$ cell count before apheresis was 18.0/µL (0–158/µL). After mobilization with only G-CSF in MM cases, the $pCD34^+$ cell count was significantly higher than in HL and NHL cases. The $pCD34^+$ cell count in MM cases was significantly higher than in NHL cases (p = 0.001) and was almost significantly different from that in HL cases (p = 0.074) (Figure 1**)**. No difference was found in $pCD34^+$ cell counts after mobilization with either cyclophosphamide or plerixafor.

After groups were classified by age into different decades, no statistically significant difference was found in $pCD34^+$ cell count. However, comparisons based on BMI revealed significantly higher $pCD34^+$ cell counts in overweight individuals compared to those of normal body weight (28 vs. 17, p = 0.014) and those who were underweight (28 vs. 14, p = 0.034) (data not shown).

Apheresis

Apheresis was not performed on four patients due to the insufficient $pCD34^+$ cell count. Among the remaining 163 patients, 245 apheresis procedures were conducted regardless of mobilization regimens. The total blood volume collected during the procedure was 4,848 ± 1,166 mL. The processed blood volume was 11,166 ± 2,145 mL. The leukocyte counts before apheresis were 30.46 × 10^9/L. The total $CD34^+$ cell count obtained post-apheresis was 3.3 × 10^9/kg, and the post-apheresis mononuclear cell (MNC) count was 49 × 10^8/kg. No correlation was found between age and collected $CD34^+$ cell count (Supplementary Figure 1). Groups were then cumulatively evaluated regardless of mobilization regimen. There was a weak negative correlation between the pre-apheresis leukocyte count (32.84 ± 13.18 x 10^9/L) and the collected $CD34^+$ cell count (2.4 ± 1.2 × 10^9/kg) (p = 0.054, r = –0.467) in the group with a $pCD34^+$ cell count of 20–50/µL on the second day of apheresis.

Transplantation and Engraftments

In 14 patients, collected cells were not utilized because they were intended solely for storage, or the patients no longer indicated for AHSCT. Successful AHSCT was performed on the remaining 144 patients (86.2%). Cell collection was unsuccessful in nine patients (5.3%); products from the other 14 patients who did not undergo transplantation were frozen and stored. Depending on disease type and status during AHSCT, patients received one of three conditioning regimens: high-dose melphalan (140–200 mg/m^2) (n = 76), BEAM (n = 67), or high-dose ICE (n = 1).

Four product bags were infused per patient on average. Infusion was performed in a single day for 122 patients with relatively lower product volumes but required two days for the remaining 22 patients with larger volumes. On average, 69.21% of the $CD34^+$ cells counted before freezing (with a maximum loss of 60% and a minimum loss of 0.5%) were available for infusion after thawing.

Neutrophil engraftment and platelet engraftment occurred on days 8–15 and days 7–16, respectively. Across regimens, for neutrophil and platelet engraftment occurred on days 11.1, and 11.2, 10.4 and 10.9, and 12 and 15 for those receiving melphalan, BEAM conditioning, and ICE, respectively.

The number of $CD34^+$ cells infused into patients negatively correlated with the dates of neutrophil and platelet engraftment (respectively, $p = 0.074$, $r = -0.150$; $p = 0.055$, $r = -0.178$). Similarly, the percentage loss of collected and infused $CD34^+$ cells positively correlated with the days of neutrophil and platelet engraftment (respectively, $p = 0.057$, $r = 0.16$; $p = 0.15$, $r = -0.132$). The insignificant effect of $CD34^+$ cell counts on neutrophil and platelet engraftments is shown in Figure 2.

DISCUSSION

ASCT remains an integral therapeutic strategy for various hematologic malignancies; its success depends on the effective mobilization of hematopoietic stem cells from the bone marrow to the peripheral blood. Over the years, several mobilization agents and strategies have been developed, primarily G-CSF, chemotherapy, and plerixafor. There is no consensus on which mobilization regimen should be used. Thus, many studies use G-CSF alone or in combination regimens. By comparing the effects of G-CSF and chemotherapy plus G-CSF on mobilization in 223 patients with MM, Sarici et al. found that G-CSF alone is affordable and sufficient (10). Dhakal et al. showed that combination therapies like G-CSF and chemotherapy with G-CSF and plerixafor achieve better mobilization but without enhancing transplant outcomes (11). In our study of a homogeneous cohort of 167 patients, mobilization was achieved using G-CSF alone in most MM cases and in at least two-thirds of lymphomas. For cases that could not be mobilized with G-CSF alone, successful mobilization was achieved in at least half of the cases using chemotherapy and G-CSF or plerixafor as alternatives.

Some patients present insufficient mobilization and are categorized as "poor mobilizers." Several factors have been identified that contribute to this clinical challenge (12) such as the type of hematologic malignancy; multiple myeloma increases the risk of poor mobilization. Prior exposure to intensive therapies can also affect mobilization outcomes. For instance, individuals who have undergone high-dose chemotherapy or radiation might experience bone marrow damage, which increases the risk of insufficient HSC mobilization. Additionally, bone marrow reserves generally decrease with age, which may hinder mobilization in older individuals. The specific mobilization protocols and agents used can also influence the outcome. Variability in response to agents like G-CSF or plerixafor has been well-documented. For example, the rate of mobilization failure was 24.1% in patients with lymphoma (13); individuals with elevated platelet counts prior to apheresis and those undergoing chemotherapy-based mobilization had a higher success rate during the initial apheresis. However, sex, age, weight, chemotherapy regimen, radiotherapy, or type of lymphoma did not significantly affect mobilization outcomes (13). Elucidating these risk factors is a pre-requisite for developing patient-specific strategies that optimize mobilization, and ultimately improve the success rates of transplantation. In our study, mobilization success decreased as the number of chemotherapy cycles increased, but this was not observed for radiotherapy. This may be because most cases received radiotherapy outside of the pelvis. Disease-specific features suggest that effective mobilization in MM cases can be attributed not due to their relapsed/refractory disease status like the lymphoma cases, but rather due to them being less affected after primary treatment. No relationship was found between patients' body weights and indices with mobilization and HSC collection. It was unclear which BMI index should be used, as HSCs can be successfully collected in obese patients. Age was not considered a negative factor in our study group.

The success of AHSCT relies on effective engraftment. Notably, the dose of infused $CD34^+$ cells has consistently been linked to faster neutrophil and platelet recovery. A higher dose of $CD34^+$ cells generally accelerates engraftment, diminishing the period of post-transplant neutropenia and thus reducing the risk of infection (14). The mobilization strategy initiated prior to AHSCT also affects treatment outcomes. Agents such as G-CSF, chemotherapy, and plerixafor can impact mobilization success and therefore the engraftment process. For instance, chemomobilization, despite its potential toxicities, often increases yields of $CD34^+$ cells compared to growth factor mobilization alone (13). Patients in whom abundant mobilization of $CD34^+$ cells is achieved are called "super-mobilizers" and demonstrate improved engraftment and survival (15). Furthermore, the patient's underlying disease and treatment history can influence engraftment outcomes. Those with a history of extensive chemotherapy or who have received multiple lines of treatment may experience delayed engraftment. Similarly, factors such as patient age, performance status, and disease stage at the time of transplant can affect engraftment speed and success (16). Lastly, conditioning regimen intensity, whether myeloablative or reduced intensity, can influence engraftment kinetics. Myeloablative regimens, while more aggressive, may accelerate and enhance engraftment compared to their reduced-intensity counterparts (17). While ASCT benefits patients with multiple myeloma and lymphoma, the engraftment process is influenced by both treatment-related and patient-specific factors. Considering all these factors can improve post-transplant outcomes and patient prognosis. In our study, the loss of $CD34^+$ cells extended the engraftment time. The initial apheresis procedure greatly determines efficacy. Thus, the timing and effective $CD34^+$ cell yield per unit volume are critical. Our study showed that efficacy wanes in subsequent procedures. Although leukocyte count increases in sequential procedures, the number of $pCD34^+$ cells does not, reducing efficacy and the amount of $CD34^+$ cells.

Our study has several limitations. First, the retrospective design inherently restricted our ability to link cause and effect. The limited patient cohort size precludes any meaningful subgroup analysis among different disease types. Furthermore, the low number of patients who underwent mobilization with plerixafor as well as those categorized as poor mobilizers or those who experienced mobilization failure rendered any statistical evaluation infeasible.

CONCLUSION

In conclusion, clinicians should strive to achieve successful mobilization on the first day of the apheresis and to direct patients to AHSCT at an appropriate time point that avoids excessive chemotherapy cycles. Successful cell collection and AHSCT can be achieved in obese and elderly patients. Future studies should be performed in larger prospective cohorts to explore engraftment kinetics.

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Ethics Committee Approval: This study was approved by the Demiroglu Bilim University Clinical Research Ethics Committee on April 11, 2013, with number 44140529/2013-036.

Informed Consent: Signed consent was obtained from the participants.

Peer-review: Externally peer-reviewed.

Authors' Contributions: Conception/Design of Study – N.B.H., M.A.; Data Acquisition, Performing experiments, Data Analysis/Interpretation and Statistical Analyses – N.B.H.; Drafting Manuscript – N.B.H, M.A.; Critical Revision of Manuscript – M.A.

Conflicts of Interests: The authors declare that they have no competing interests.

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Clinical Trial Registration: The authors report that this study is not a clinical trial.

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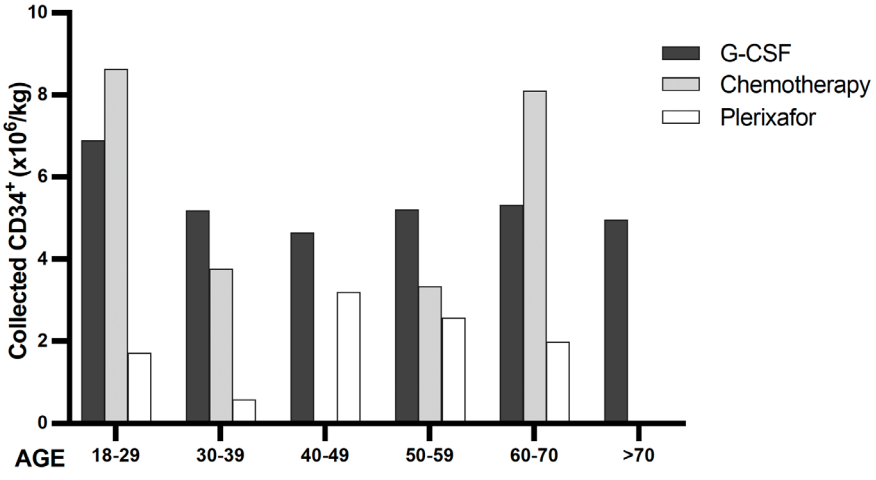
Supplemantary Table 1. Formulas used in the study.	
Metric (Unit)	**Formula**
BMI	Weight / [height in meters]^2 (kg/m^2)
IBW	Female; 50 + [0.91 x (height in cm- 152)] (kg)
	Male; 45 + [0.91 x (height in cm- 152)] (kg)
ABW	IBW + 0.4 x (body weight- IBW) (kg)
AdjIBW	IBW + [0.25 x (ABW- IBW)] (kg)

BMI, body mass index; IBW, Ideal Body Weight, ABW, Actual Body Weight , and AdjIBW, Adjusted Ideal Body Weight. Patients were classified based on their body mass index (BMI) as underweight (below 20 kg/m^2), normal weight (20-25.9 kg/m^2), overweight (26-29.9 kg/m^2), and obese (above 30 kg/m^2).



Supplementary Figure 1. Distribution of collected CD34$^+$ cell counts