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DOES REINFUSION OF STEM CELL PRODUCTS ON MULTIPLE DAYS AFFECT ENGRAFTMENT?

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Abstract

Introduction: High dose melphalan chemotherapy and autologous stem cell transplantation in multiple myeloma (MM) is still important treatment modality in transplant eligible patients. At least 2 x 10^6 /kg CD 34+ cell dose is preferred for sufficient engraftment. Some patients need multiple leukapheresis procedures to reach a required number of the CD34+ cell but this can cause high volume of stem cell product that can not be given in a single day.

Aim: Whether the number of infusion days affect engraftment or not, isn't studied before. We want to evaluate the effect of reinfusion of stem cell in multiple day on engraftment results. Demographic features, CD 34+ cell doses, neutrophil and platelet engraftment days, hospitalization days, number of infusion days of 149 autologous transplantation of 143 multiple myeloma patients were evaluated retrospectively.

Results: The data of 143 multiple myeloma patients who were transplanted were analyzed retrospectively. Median age was 55 ± 8,5 (26-70) with 91/58 Male/Female (M/F) ratio. The hospitalization day for all patients was 24 ± 6 (14-50) day. Mean CD 34+ cell number was 7,5 ±5,3 x 10^6/kg(1,5-31 x 10^6/kg). CD34+ cells were reinfused in one day in 80,5% (n:120) of the patients, 2 days in 18,2% of the patients (n:27) and3 days in 1,3% of patients (n:2). For 29 patients, reinfusion was performed in more than one day because of the high volume of stem cell product. We didn't see any dimethyl sulfoxide toxicity, cardiac arrhythmia and volume overload complication. Hypertensive attack during infusion was easily controlled by frusemide infusion. In multiple infusions group, the infused CD34+ cell numbers were mean 4,8 ± 2,8x10^6/kg and in single infusion group, the infused CD34+ cell numbers were mean 8,1 x10^6/kg ± 5,5 x10^6/kg. There were no statistical differences between two groups regarding to platelet and neutrophil engraftment days (p= 0.85 and p= 0.5). There was no statistical difference between two groups for the hospitalization days (p=0.06).

Conclusion: In cases with high volume stem cell product to obtain sufficient stem cell, reinfusion can be safely applied over several days without any delay in engraftment.

Keyword: Multiple myeloma, autologous transplantation, multiple reinfusion day.

Introduction:

Multiple myeloma (MM) is a B-cell malignancy with a median age at presentation of 60–65 years (1). High dose melphalan chemotherapy and autologous stem cell transplantation (ASCT) in multiple myeloma is still important treatment modality in transplant eligible patients (2). Advanced age is a poor prognostic factor in trials using conventional chemotherapy even if the biological and clinical features in elderly MM patients are identical to those of younger patients (3,4). This procedure is safe even in selected patients older than the age of 65. Because usage of peripheral stem cell shortened the period of hematopoietic recovery leading to a significant decrease in mortality and morbidity. ASCT has been a part of myeloma treatment in these patient group (5, 6, 7). Adequate collection and administration of sufficient CD34+ hematopoietic stem cells are needed for the successful transplantation. Establishing an appropriate minimum CD34+ cell dose for MM patients aged 65 years and older is critical given that the median age of diagnosis for MM is generally in between 65 and70 years of age and many of these patients may be transplant eligible (8). Recently practice, at least 2 x 106 /kg CD 34 dose is preferred for sufficient neutrophil and platelet engraftment, and ≥ 5x 106 /kg CD34+ is associated with a shortened time to platelet recovery (9,10).

The aim of our study was to examine the effect of CD34+ reinfusion in multiple days on engraftment results in MM patients. We were evaluated the demographic features, infused CD 34+ cell doses, neutrophil and platelet engraftment days, hospitalization days and the number of infusion days in 149 ASCT of 143 multiple myeloma patients retrospectively, .

Materials and Methods:

Between February 2009 and September 2017, 143 patients with MM underwent ASCT at Dokuz Eylul University Hospital, Division of Hematology. Data were collected from the electronic and patient files medical archives retrospectively. Baseline patient characteristics are shown in Table 1. All patients were suitable for the ASCT therapy and had enough stem cell collection. However, second ASCT were planned in six patients due to late relapse of the disease. All patients were informed about the benefits and risks associated with stem cell collection and transplantation. The majority of the patients had one or two line of prior chemotherapy (range:1-3) at pre-ASCT period (Vincristine -Adriablastin-Dexamethasone (VAD) therapy, Bortezomib - Dexamethasone therapy and Lenalidomide – Dexamethasone therapy).

Transplant details including mobilizing agents, CD 34+ cell doses, neutrophil and platelet engraftment days, hospitalization days, number of CD34+ reinfusion days were analyzed. The data was examined according to the number of CD34+ cell reinfusion days. The neutrophil and platelet engraftment days, hospitalization days and collected CD34+ cell count of the patients whom CD34+ cell been reinfused in one day was compared to that of the patients whom CD34+ cell been reinfused in multiple day. At the time of transplant, only 11,4% (n:17) of patients were in complete response (CR). The majority (n:86, 57,6%) had reached a very good partial response. 28% (n:42) of the patients had reached a partial response (PR) and 3% (n:4) had the refractory or progressive disease. Second autologous transplantation was planned in six patients because of progressive disease.

Peripheral blood stem cells (PBSC) were collected in 1-4 apheresis procedure (mean:1,7), following mobilization regimens. We used cyclophosphamide 2,4 g/m2 IV. for one day with MESNA and granulocyte colony stimulating factor (G-CSF, 5 mcg/kg/day sc) in 133 patients (89,3%), G-CSF alone in 7 patients (4,7%) and plerixafor plus G-CSF in 9 patients (6%) for mobilization. Apheresis was initiated upon recovery of CD34+ cells to 10 >µL. Each sample was investigated by flow cytometric analysis for the presence of cells expressing CD34. The minimum target CD34+ stem cell dose for collection was > 2x106 CD34/kg for each autologous transplantation.

The conditioning regimen consisted of melphalan in all patients. Melphalan was given at a dose of 200 mg/m2 in 124 patients (83,2%) and at reduced dose 140 mg/m2 in 25 patients (16,8%) due to reduced creatinine clearance (< 50 ml/min). Patients received G-CSF once a day starting on day 1 after the infusion of stem cells until the time of engraftment.

Statistical Analysis:

Descriptive statistics were used for baseline characteristics, transplant- related factors and posttransplant results. Differences in the distribution of variables between patient subsets were analyzed using Pearson x2 test/ correlation test/ t-test. Response rate the patient was assessed using the McNemar test for paired categorical variables. All statistical analyses were two-sided tests with 0.05 as the critical level of significance, and p values were reported.

Results:

We analyzed 149 autologous transplantation of 143 multiple myeloma patients between February 2009 and September 2017 retrospectively. Median age was 55 ± 8,5 (26-70) with 91/58 M/F ratio. There were no significant differences in platelet engraftment days, neutrophil engraftment days, reinfusion days, hospitalization days and infused CD34+ dose with regards to gender distribution (Table 2).

Patients were divided into two age groups, aged under 60 years and older. There was no significant difference between the two groups in terms of the platelet and neutrophil engraftment days, multiple day reinfusion rate, hospitalization days and infused CD34+ cell dose (Table 3).

When we analyzed all patients, the hospitalization days were 24 ± 6 (14-50) . Mean CD 34+ cell count was 7,5 ±5,3 x 106/kg (1,5-31 x 106/kg). The platelet engraftment days were found 13,9 ± 3 (9-30) and the neutrophil engraftment days were found 11,5 ± 1,5 (8-17).

The higher reinfused CD34+ cell doses were associated with faster platelet and neutrophil engraftment (p=0.034 and p= 0.001). The hospitalization days had shortened because of a better transplantation outcome took with the higher reinfused CD34+ cell doses (p=0.001).

CD34+ cells were reinfused in one day in (n:120) 80,5% of patients, 2 days in 18,2% of patients(n:27) and 3 days in 1,3% of patients (n:2). For 29 patients, reinfusion was performed in more than one day, because of the higher volume of stem cell product and according to the tolerability of the patients. However, reınfusion of PBMC cryopreserved with DMSO can associated with toxic reactions. We know that infusion of product containing more than 1 g / kg of DMSO per day can lead to increased DMSO toxicity. As a result, the days of reinfusion were determined according to the performance status of our patients, the amount of product they had and the amount of DMSO contained in the products. There were also statistical differences between two groups in mobilization days. The mobilization days were found higher in the multiple day infusion group than in the single day infusion group (2,2 day x 1,6 day, p= 0.0001). We didn't see any dimethyl sulfoxide toxicity, cardiac arrhythmia and volume overload complications. Hypertensive attack during infusion was easily controlled by frusemide infusion. In two groups, CD 34+ cell levels were mean 4,8 ± 2,8x106/kg (in multiple day infusions group) and 8,2 x106/kg ± 5,5 x106/kg (in single day infusion group). The infused CD 34+ cell count was found higher in the single day infusion group than in the multiple day infusion group (p=0.003). There were no statistical differences between two

groups in case of platelet and neutrophil engraftment days (p= 0.85 and p= 0. 5) and also for hospitalization days (p=0.06) (Table 4).

Discussion:

Multiple Myeloma is a disease of the elderly. ASCT is the important treatment modality in symptomatic myeloma patients. Treatment options have expanded in the last decade with novel drugs. But ASCT still maintains its place in the treatment of myeloma. We present the results of a retrospective analysis of MM patients with autologous transplantation in our center at last decade. The median age was 55 years and there was male gender dominance in our study. The median age was being relatively young in our study, similar to the study of Terpos et al. (11).

The recent population-based studies have shown increasing use of ASCT in elderly patients with MM (12). However, different age cut-off values as 60 years, 65 years or 70 years were given that estimates independently for survival in different studies (13, 14).

In our study, ASCT was planned in the transplant-eligible patients who had adequate stem cell collection. We applied cyclophosphamide and G-CSF as mobilization regimen mostly. The target CD34+ stem cell dose for the collection was > 2x106 CD34/kg for each autologous transplantation. The mean CD 34+ cell number was 7,5 ±5,3 x 106/kg in our study. The mean platelet engraftment days were found 13,9 and the mean neutrophil engraftment days were found 11,5 in our study. In other studies, the median time to neutrophil engraftment and the median time to platelet engraftment were reported as 9 -14 days (15) and 13, 5 - 25 days (16) respectively. The prior studies used the total infused CD34+ cells as a predictor of neutrophil and platelet engraftment. We also demonstrated a significant correlation between the infused CD34+ cell dose and the time to platelet egraftment and neutrophil engraftment (17, 18, 19, 20).

The collected stem cell product is mostly given as single day infusion but in some situations, the product can be reinfused in multi-day due to patient characteristics or concern about higher volume-related complications.

We chose multi-day reinfusion to overcome possible volume overload in the older patient. DMSO toxicity could be another problem in the patient if given in a single day. Patients requiring multiple day to collect an adequate number of CD34+ cell may be at risk of exposure to serious doses of DMSO. Davis et al have suggested that toxicities related to the infusion of cryopreserved cells due to related to the volumes of cryoprotectants (21). But our study didn't demonstrate a difference in toxicity with multiple day infusion like the study of Abdel Razeq et al (22). We didn't see any another toxicity as cardiac arrhythmia, volume overload complication. We also wondered the effect of multi-day infusion on the engrafment. The effect of multi-day infusion of stem cells on engraftment was evaluated in the study of Abdel Razeq et al. They showed there was no effect on regarding engrafment. However, this study consisted of a heterogeneous group of the patients with non-hodgkin lymphoma, hodgkin lymphoma and breast cancer, did not include the myeloma patient. There is no another study evaluating effect of multi-day stem cell infusion on engraftment in the

literature. If we consider all patients in our study, there were no statistical differences between two groups (multiple reinfusion days and single reinfusion day) regarding platelet and neutrophil engraftment days and hospitalization days.

We also observed that the multi-day infusion of stem cells due to higher number of package mostly used in the patient older than 60 year. The age of these patients was between 26 and 70 years. 39% of them were older than 60 years and 61% were younger than 60. On the other hand, the number of patients older than 65 years old was found to be 14%. In the study of GITMO-WG, age over 65 was described as poor mobilizing factor like previous cytotoxic chemotherapy, radiotherapy, bone marrow involvement and platelet count before mobilization (23). We did not observed any problem in the mobilization of myeloma patients regarding the age.

In our study, we also compared this in between two age groups (<60 year-old, ≥60 year-old). There were no significant differences in platelet engraftment days, neutrophil engraftment days, reinfusion days, hospitalization days and infused CD34+ dose in these two groups. But Larysa et al were found a significant difference in mean hospitalization days (18,6 days in older versus 16,8 days in younger, $p< 0.01$) and no significant difference in hospital mortality between elderly and younger patients (24).

Conclusion: In cases with high volume stem cell product to obtain sufficient stem cell, reinfusion can be safely applied over several days without any delay in engraftment.

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Compliance with Ethical Standards

Conflict of Interest The author did not receive financial compensation for authoring the manuscript.

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Table 1. Patients characteristics

	Number (n)	%
Patients	143	100
Age (years) (median, range)	55 (26-70)	
Gender (Male/Female)	91/58	61/39
Status at transplantation		
CR	17	11,3
VGPR	86	57,7
PR	42	28
Progressive / Refractory	4	3
Mobilization Regimens		
Cyclophosphamide/ G-CSF	133	89,3
Plerixafor/ GCSF	9	6
G-CSF	7	4,7
Conditioning		
Melphalan 200mg/m^2	124	83,2
Melphalan 140mg/m^2	25	16,8
Death	37	24,8

Abbreviations: CR= complete remission, VGPR= very good partial remission, PR= partial remission, G-CSF= Granulocyte coloni stimilan factor

Table 2. Results regards to gender distribution.

	Male	Female	P value

Patient number (n/%)	91 / 61	58 / 39	
Platelet engraftment day	14 ± 3,4	14± 3,6	0.7
Neutrophil engraftment day	11,7 ± 1,6	11,3 ± 1,3	0.15
Reinfusion days	1,2 ± 0,4	1,1 ± 0,4	0.33
Infused CD34+ dose	7,5±5,2 x10^6 CD34/kg	7,4±5,3 x10^6 CD34/kg	0.8
Hospitalization day	24 ± 5	24 ± 6	0.53

Table 3. Results regards to age (<60 years and ≥ 60 years)

	<60 years	**≥ 60 years**	**P value**
Patient number (n/%)	91 / 61	58 / 39	
Platelet engraftment day	14 ± 3,7	13,7 ± 2,9	0.56
Neutrophil engraftment day	11,5 ± 1,4	11,5 ± 1,6	0.9
Reinfusion days	1,1 ± 0,3	1,2 ± 0,4	0.26
Infused CD34+ dose	7,7±5,3 x10^6 CD34/kg	7,1±5,2 x10^6 CD34/kg	0.5
Hospitalization day	24 ± 6,4	23,5 ± 4,7	0.31

Table 4. Neutrophil, platelet engraftment and hospitalization daytime and infused CD34+cell according to reinfusion days

	Multiple infusions group	**Single infusion group**	**P value**
Patient number (n/%)	29 / 19,5	120 / 80,5	
Platelet engraftment day	14,3 ± 3,2	13,7 ± 3,5	0.85
Neutrophil engraftment day	11,9 ± 1,4	11,5 ± 1,5	0.5
Infused CD34+ dose	4,8 ± 2,8 x10^6 CD34/kg	7,1±5,2 x10^6 CD34/kg	**0.003**
Hospitalization day	26 ± 7	23,5 ± 5	0.06