

# Analysis of Factors that Influence Hematopoietic Recovery in Autologous Transplanted Patients with Hematopoietic Stem Cells from Peripheral Blood

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## Abstract

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**BACKGROUND:** Successful hematopoietic stem cell transplantation (HSCT) requires a rapid and durable hematopoietic recovery.

**AIM:** The aim of our study was to analyse factors that influence hematopoietic recovery after autologous HSCT.

**MATERIALS AND METHODS:** Multiple regression analysis was used to analyse factors affecting neutrophil and platelet engraftment in 90 autologous transplanted patients – 30 with acute myeloid leukaemia (AML), 30 with lymphoma and 30 with multiple myeloma (MM) from 2008 till 2016.

**RESULTS:** The neutrophil recovery in AML patients was significantly influenced by transfusion support with random-donor platelets, sex and number of transplanted mononuclear cells (MNC) and CD34+ cells; and in lymphoma patients, it was influenced by sex, age, mobilisation strategy and some transplanted MNC. The influence of investigated factors on neutrophil engraftment in MM patients was not statistically significant. The platelet recovery in AML patients was influenced by transfusion support with random-donor platelets; in lymphoma patients, it was influenced by sex, age, time from diagnosis to harvesting and time from diagnosis to HSCT; and in MM patients it was influenced by transfusion support with random-donor platelets.

**CONCLUSION:** Additional studies are necessary to better understanding of engraftment kinetic to improve the safety of HSCT and to minimise potential complications and expenses related to HSCT.

## Introduction

Hematopoietic stem cell transplantation (HSCT) is an established treatment for patients with life-threatening haematological and non-hematological diseases, including acute and chronic leukaemias, myelodysplastic syndromes, lymphomas, multiple myeloma, aplastic anaemia, immune disorders, and congenital disorders of metabolism [1-6]. This therapy consists of the intravenous infusion of hematopoietic progenitor cells to reestablish marrow function in patients with damaged or defective bone marrow [7]. Transplantation of hematopoietic stem cells (HSC) provides complete and long-term reconstitution of the hematopoietic system of the patients [8, 9] and may result in remission or cure in a proportion of cases [1]. Given the numerous advantages such as, lower rates

of morbidity and mortality, shorter hospitalization, lower costs of treatment and the possibility of applying high-dose chemotherapy to an older group of patients, mobilized peripheral blood stem cells have largely replaced the use of bone marrow-derived stem cells as the preferred source of hematopoietic stem cells in autologous transplantation [7-15].

Furthermore, the use of granulocyte-colony-stimulating factor (G-CSF) mobilized peripheral blood stem cell transplantation instead of bone marrow, may reduce the rates of graft rejection, enhanced engraftment and accelerated hematopoietic recovery [7, 16]; because peripheral blood stem cell transplantation affords about 10-fold more T cells [7, 17], compared to bone marrow, and a 2-fold higher CD34+ cell dose [7, 18, 19]. Fast and durable hematopoietic recovery with absolute neutrophil count (ANC) > 0.5x10<sup>9</sup>/l and platelets (Plt) > 20x10<sup>9</sup>/l

after reconstitution is an imperative for successful hematopoietic stem cell transplantation. Rapid hematopoietic recovery at autologous peripheral stem cell transplantation is a significant factor in the reduction of early transplant related complications and costs [20]. It is very important to recognise the factors that influence the hematopoietic recovery after peripheral blood stem cell transplantation.

The aim of our study is to analyse factors that influence the quality of graft through hematopoietic recovery in autologous transplanted patients with peripheral blood hematopoietic stem cells.

## Materials and Methods

This is a retrospective-prospective study performed at the Institute for Transfusion Medicine of RM and University Clinic of Hematology in Skopje, Macedonia from 2008 till 2016. The investigated group consisted of 90 hematologic patients – 30 patients diagnosed with acute myeloid leukaemia (AML), 30 with lymphoma and 30 with multiple myeloma (MM). The study was approved by the Ethical Committee for Biomedical Research at Medical Faculty in Skopje. All subjects in the study gave their written consent for performing the research, mobilising strategy, apheresis collection of hematopoietic stem cells from peripheral blood, cryopreservation and transplantation (according to Recommendations of WMA Revision of Declaration of Helsinki). Mobilisation of HSC is performed with the granulocyte colony-stimulating factor (G-CSF) 10 µg/kg/day (as a single mobilising strategy) or in a combination of G-CSF + chemotherapy depending on diagnosis and disease status. The collection of HSC was performed by the apheresis procedure with the cellular separator COBE Spectra Version 6.1 (TerumoBCT) in the Institute for Transfusion Medicine of RM, processing 2-2.5 total blood volumes [9]. The minimum number of HSC to be collected was  $\geq 2 \times 10^8$ /kg MNC and  $\geq 2 \times 10^6$ /kg CD34+ cells. Cryopreservation and transplantation of HSC were performed at the University Clinic of Hematology. Irradiated and filtered blood products were given to maintain the haemoglobin level above 8 g/dl and the platelet count above  $10 \times 10^9$ /l [21, 22]. The quality of hematopoietic stem cell grafts collected in our study was determined through their clinical efficiency in hematopoietic recovery after autologous transplantation. The number of CD34+ cells in the graft [23], the number of mononuclear cells (MNC) in the graft and the time to neutrophil and platelet engraftment were determined at the University Clinic of Hematology. Neutrophil engraftment was defined as the first of the three consecutive days post-transplant where absolute neutrophil count reached >

$0.5 \times 10^9$ /l and platelet count >  $20 \times 10^9$ /l, unsupported by transfusion and G-CSF more than five days from the first increase in the investigated parameters.

## Statistical analysis

Statistical analysis was done in the statistical program Statistica 7.1 and SPSS 17.0. The following methods were used in this study: the percentage of structure (%) was determined in series with attributive marks; descriptive statistics (Mean  $\pm$  Std.Dev., Minimum, Maximum, Median) was used in series with numerical marks; differences between the autologous subgroups of patients (AML, lymphoma and MM) in the parameters with attributive and numerical marks were tested with Analysis of Variance ANOVA test; the influence of possible predictive factors on hematopoietic recovery was determined by the multiple regression analysis. The accepted level of significance was  $p < 0.05$ .

## Results

### Characteristics of patients

Characteristics of autologous transplanted patients with AML, lymphoma and MM are shown in Table 1.

**Table 1: Clinical characteristics of autologous transplanted patients with AML, lymphoma and MM**

Patients characteristics	AML patients		Lymphoma patients		MM patients	
	Number	%	Number	%	Number	%
Sex						
Men	17	56.7	20	66.7	19	63.3
Women	13	43.3	10	33.3	11	36.7
Age mean, std. dev, range)	44.5 $\pm$ 13.7 (18-65)		37.6 $\pm$ 12.0 (18-57)		53.1 $\pm$ 7.8 (39-65)	
< 20 years	1	3.3	2	6.7	/	/
20-29	5	16.7	6	20.0	/	/
30-39	4	13.3	9	30.0	2	6.7
40-49	6	20.0	7	23.3	8	26.7
50-59	12	40.0	6	20.0	13	43.3
$\geq 60$	2	6.7	/	/	7	23.3
Disease stage						
CR	30	100.0	6	20.0	10	33.3
PR	/	/	6	20.0	17	56.7
Relapse	/	/	18	60.0	3	10.0
Chemotherapy cycles						
0						
1-4	28	93.3	1	3.3	16	53.3
5-8	2	6.7	11	36.7	8	26.7
9-12			10	33.3	3	10.0
13-16			5	16.7	2	6.7
$\geq 17$			3	10.0	1	3.3
Time from diagnosis to harvesting (months)	4.8 $\pm$ 1.9 (range 1-11)		33.2 $\pm$ 41.1 (range 5-148)		12.5 $\pm$ 15.2 (range 2-79)	
Time from diagnosis to HSCT	5.8 $\pm$ 1.8 (range 3-12)		34.5 $\pm$ 41.4 (range 5-150)		12.8 $\pm$ 15.2 (range 2-79)	

AML= acute myeloid leukaemia; MM = multiple myeloma; CR = complete remission; PR = partial remission; HSCT = hematopoietic stem cell transplantation.

### Number of transplanted peripheral blood hematopoietic stem cells

The mean number of transplanted MNC and CD34+ cells in AML patients was  $2.86 \pm 1.18$  (range

0.9-5.8) and  $2.62 \pm 1.04$  (range 0.9-5.5) respectively. The mean number of transplanted MNC and CD34+ cells in lymphoma patients was  $3.18 \pm 1.07$  (range 0.8-5.6) and  $2.99 \pm 0.94$  (range 0.7-5.0) respectively. The mean number of transplanted MNC and CD34+ cells in MM patients was  $3.23 \pm 1.16$  (range 1.0-6.2) and  $2.95 \pm 1.18$  (range 0.9-5.9) respectively. There was not statistically significant difference in the number of transplanted MNC and CD34+ cells between the autologous patients with AML, lymphoma and MM for  $p > 0.05$  ( $F = 0.785031$ ,  $p = 0.459309$ ;  $F = 1.059888$ ;  $p = 0.350925$ ).

### Engraftment

Neutrophil engraftment ( $ANC > 0.5 \times 10^9/l$ ) occurred mean on day  $12.8 \pm 3.2$  (median 12) in AML patients (range 7-22); mean on day  $12.1 \pm 4.8$  (median 11) in lymphoma patients (range 7-25) and  $12.2 \pm 2.7$  (median 11) with range 8-19 in MM patients (Table 2). There was not statistically significant difference in the neutrophil engraftment time between AML, lymphoma and MM patients for  $p > 0.05$  ( $p = 0.787076$ ).

**Table 2: Neutrophil engraftment ( $ANC > 0.5 \times 10^9/l$ ) and platelet engraftment ( $Plt > 20 \times 10^9/l$ ) in autologous transplanted patients with AML, lymphoma and MM**

$ANC > 0.5 \times 10^9/l$	Mean	Median	Minimum	Maximum	Std.Dev
AML patients	12.8	12.0	7.0	22.0	3.15
Lymphoma patients	12.1	11.0	7.0	25.0	4.80
MM patients	12.2	11.0	8.0	19.0	2.74
$Plt > 20 \times 10^9/l$	Mean	Median	Minimum	Maximum	Std.Dev
AML patients	15.8	14.5	7.0	29.0	5.73
Lymphoma patients	15.3	13.0	8.0	38.0	7.45
MM patients	14.2	12.5	7.0	32.0	4.97

ANC = absolute neutrophil count; Plt = platelets; N = number; Std. Dev.= standard deviation.

Platelet engraftment ( $Plt > 20 \times 10^9/l$ ) occurred mean on day  $15.8 \pm 5.7$  (median 14.5) in AML patients (range 7-29); mean on day  $15.3 \pm 7.5$  (median 13) in lymphoma patients (range 8-38) and  $14.2 \pm 5.0$  (median 12.5) with range 7-32 in MM patients (Table 3). There was not statistically significant difference in the platelet engraftment time between AML, lymphoma and MM patients for  $> 0.05$  ( $p = 0.588418$ ).

**Table 3: Transfusion support in autologous transplanted patients with AML, lymphoma and MM**

Transfusion support	Number (%)	Mean	Minimum	Maximum	Std. Dev.
<b>AML patients</b>					
Erythrocytes	20 (66.7%)	2.95	1	6	1.61
Random donor platelets	30 (100%)	35.10	12	93	19.40
Single donor platelets	5 (16.7%)	2.00	1	5	1.73
Fresh frozen plasma	28 (93.3%)	4.82	1	8	1.81
<b>Lymphoma patients</b>					
Erythrocytes	25 (83.3%)	3.52	2	12	2.30
Random donor platelets	30 (100%)	24.23	3	108	21.10
Single donor platelets	4 (13.3%)	1.00	1	1	0.00
Fresh frozen plasma	30 (100%)	5.63	2	9	1.69
<b>MM patients</b>					
Erythrocytes	18 (60%)	2.28	1	4	0.83
Random donor platelets	27 (90%)	27.33	3	140	26.96
Single donor platelets	3 (10%)	1.00	1	1	0.00
Fresh frozen plasma	28 (93.3%)	2.89	1	32	5.75

Note: 4-6 random donor platelet units make one adult therapeutic dose (ATD); one single donor platelet unit (apheresis platelet unit) is one ATD.

Transfusion support with erythrocytes, random-donor platelets, single-donor platelets and fresh frozen plasma in autologous transplanted AML, lymphoma and MM patients is shown in Table 3.

The biggest consumers of erythrocyte transfusions were lymphoma patients, the biggest consumers of platelet transfusions were AML patients and the biggest consumers of FFP were lymphoma patients. MM patients received less transfusion than the other two subgroups (AML and lymphoma patients).

### Multiple regression analysis

The relationship between the neutrophil engraftment ( $ANC > 0.5 \times 10^9/l$ ) in autologous transplanted patients with AML (dependent variable) and investigated possible predictive variables of interest was investigated with the multiple regression analysis.

**Table 4: Multiple regression analysis of neutrophil engraftment ( $ANC > 0.5 \times 10^9/l$ ) in autologous transplanted patients with AML**

INDEPENDENT VARIABLES	R = 0.871 F = 2.339		R <sup>2</sup> = 0.758 P = 0.025983	
	Beta	t – test	p – level	
Age	0.017334	0.10645	0.916734	
Sex	0.552950	2.81100	0.013877*	
Mobilization strategy	-0.191639	-0.54733	0.592769	
Beginning of harvesting	0.345068	0.92407	0.371101	
Therapy regimen	-0.304382	-1.23559	0.236943	
Chemotherapy cycles	-0.052788	-0.24660	0.808796	
Comorbidity	0.076792	0.28509	0.779749	
Time from diagnosis to harvesting	0.375763	1.02715	0.321772	
Time from diagnosis to HSCT	-0.749913	-1.74343	0.103167	
Number of transplanted MNC	0.707462	2.74263	0.015873*	
Number of transplanted CD34+ cells	0.779065	3.07735	0.008193*	
Transfusion of Erythrocytes	-0.020631	-0.12694	0.900792	
Transfusion of Platelets (RD)	0.552950	3.31236	0.005134*	
Transfusion of Platelets (SD)	-0.315388	-1.76382	0.099562	
Transfusion of FFP	0.188833	0.99061	0.338688	

\*statistically significant, ANC-absolute neutrophil count, HSCT-hematopoietic stem cell transplantation, MNC-mononuclear cells, RD-random-donor, SD-single-donor, FFP-fresh frozen plasma.

The analysis showed that the coefficient of multiple correlations (R) was 0.871, the coefficient of determination (R<sup>2</sup>) was 0.758 and it showed that all independent variables together influence the variability of neutrophil engraftment in this group of patients with 75.8%, while 24.2% were influenced by other factors. The significance of the coefficient of multiple correlations (R) tested by F-distribution showed that the influence of possible predictive variables on neutrophil engraftment in autologous transplanted patients with AML is statistically significant for  $p = 0.025983$ . The analysis of independent variables showed that sex (Beta = 0.552950,  $p = 0.013877$ ), number of transplanted MNC (Beta = 0.707462,  $p = 0.015873$ ), number of transplanted CD34+ cells (Beta = 0.779065,  $p = 0.008193$ ) and transfusion support with random donor platelets (Beta = 0.552950,  $p = 0.005134$ ) had a significant influence on neutrophil engraftment in autologous transplanted patients with AML as presented in Table 4.

The relationship between the platelet engraftment ( $Plt > 20 \times 10^9/l$ ) in autologous transplanted patients with AML (dependent variable) and investigated possible predictive variables of interest was investigated with the multiple regression analysis. The analysis showed that the coefficient of multiple correlations (R) was 0.880, the coefficient of determination ( $R^2$ ) was 0.775 and it showed that all independent variables together influence the variability of neutrophil engraftment in this group of patients with 77.5%, while other factors influenced 22.5%.

The significance of the coefficient of multiple correlations (R) tested by F-distribution showed that the influence of possible predictive variables on platelet engraftment in autologous transplanted patients with AML is statistically significant for  $p = 0.017603$ . The analysis of independent variables showed that transfusion support with random-donor platelets had a significant influence on platelet engraftment in autologous transplanted patients with AML (Beta = 0.403,  $p = 0.03747$ ). The influence of other predictive factors of interest was not statistically significant in this investigated group.

**Table 5: Multiple regression analysis of neutrophil engraftment ( $ANC > 0.5 \times 10^9/l$ ) in autologous transplanted patients with lymphoma**

INDEPENDENT VARIABLES	R = 0.906		$R^2 = 0.921$	
	F = 10.972		P = 0.000028	
	Beta	t - test	p - level	
Age	-0.50071	-4.57874	0.000429*	
Sex	0.30710	2.60319	0.020848*	
Mobilization strategy	-1.02503	-3.49451	0.003574*	
Beginning of harvesting	0.40853	1.60416	0.130994	
Therapy regimen	0.03980	0.40630	0.690666	
Chemotherapy cycles	-0.16572	-1.15800	0.266237	
Comorbidity	0.07835	0.66858	0.514640	
Time from diagnosis to harvesting	-3.81958	-1.26518	0.226462	
Time from diagnosis to HSCT	4.39536	1.45727	0.167105	
Number of transplanted MNC	0.41135	2.87015	0.012349*	
Number of transplanted CD34+ cells	0.27615	1.83993	0.087074	
Transfusion of Erythrocytes	0.16855	1.82848	0.088859	
Transfusion of Platelets (RD)	0.09061	0.71402	0.486949	
Transfusion of Platelets (SD)	0.10904	1.01845	0.325742	
Transfusion of FFP	0.09635	0.92769	0.369287	

\*Statistically significant, ANC-absolute neutrophil count, HSCT-hematopoietic stem cell transplantation, MNC-mononuclear cells, RD-random-donor, SD-single-donor, FFP-fresh frozen plasma.

The relationship between the neutrophil engraftment ( $ANC > 0.5 \times 10^9/l$ ) in autologous transplanted patients with lymphoma (dependent variable) and investigated possible predictive variables of interest was investigated with the multiple regression analysis. The analysis showed that the coefficient of multiple correlations (R) was 0.960, the coefficient of determination ( $R^2$ ) was 0.921 and it showed that all independent variables together influence the variability of neutrophil engraftment in this group of patients with 92.1%, while other factors influenced 7.9%. The significance of the coefficient of multiple correlations (R) tested by F-distribution showed that the influence of possible predictive variables on neutrophil engraftment in autologous transplanted patients with lymphoma is statistically

significant for  $p = 0.000028$ . The analysis of independent variables showed that age (Beta = -0.50071,  $p = 0.000429$ ), sex (Beta = 0.30710,  $p = 0.020848$ ), mobilizing strategy (Beta = -1.02503,  $p = 0.003574$ ) and number of transplanted MNC (Beta = 0.41135,  $p = 0.012349$ ) had a significant influence on neutrophil engraftment in autologous transplanted patients with lymphoma as presented in Table 5.

The relationship between the platelet engraftment ( $Plt > 20 \times 10^9/l$ ) in autologous transplanted patients with lymphoma (dependent variable) and investigated possible predictive variables of interest was investigated with the multiple regression analysis. The analysis showed that the coefficient of multiple correlations (R) was 0.913, the coefficient of determination ( $R^2$ ) was 0.839 and it showed that all independent variables together influence the variability of neutrophil engraftment in this group of patients with 83.9%, while other factors influenced 16.1%. The significance of the coefficient of multiple correlations (R) tested by F-distribution showed that the influence of possible predictive variables on platelet engraftment in autologous transplanted patients with lymphoma is statistically significant for  $p = 0.002622$ . The analysis of independent variables showed that age (Beta = 0.470,  $p = 0.009697$ ), sex (Beta = 0.333,  $p = 0.069099$ ), time from diagnosis to harvesting (Beta = 11.261,  $p = 0.021012$ ) and time from diagnosis to HSCT (Beta = 11.408,  $p = 0.019575$ ) had a significant influence on platelet engraftment in autologous transplanted patients with lymphoma.

The relationship between the neutrophil engraftment ( $ANC > 0.5 \times 10^9/l$ ) in autologous transplanted patients with MM (dependent variable) and investigated possible predictive variables of interest was investigated with the multiple regression analysis. The analysis showed that the coefficient of multiple correlations (R) was 0.833, the coefficient of determination ( $R^2$ ) was 0.694 and it showed that all independent variables together influence the variability of neutrophil engraftment in this group of patients with 69.4%, while other factors influenced 30.6%. The significance of the coefficient of multiple correlations (R) tested by F-distribution showed that the influence of possible predictive variables on neutrophil engraftment in autologous transplanted patients with MM was not statistically significant for  $p = 0.084509$ . The analysis of independent variables showed that the influence of possible predictive variables of interest is not statistically significant in this investigated group.

The relationship between the platelet engraftment ( $Plt > 20 \times 10^9/L$ ) in autologous transplanted patients with MM (dependent variable) and investigated possible predictive variables of interest was investigated with the multiple regression analysis. The analysis showed that the coefficient of multiple correlations (R) was 0.908, the coefficient of

determination ( $R^2$ ) was 0.824 and it showed that all independent variables together influence the variability of platelet engraftment in this group of patients with 82.4%, while other factors influenced 17.6%.

**Table 6: Multiple regression analysis of platelet engraftment (Plt > 20x10<sup>9</sup>/l) in autologous transplanted patients with MM**

INDEPENDENT VARIABLES	R = 0.908		R <sup>2</sup> = 0.824
	F = 4.366		
	Beta	t - test	p - level
Age	0.10858	0.50699	0.620055
Sex	-0.08515	-0.46229	0.650979
Mobilization strategy	-0.39208	-0.66121	0.519213
Beginning of harvesting	0.35460	0.59844	0.559106
Therapy regimen	-0.25366	-1.17937	0.257904
Chemotherapy cycles	0.24995	1.59500	0.133031
Comorbidity	0.38512	1.56811	0.139176
Time from diagnosis to harvesting	6.24264	1.08700	0.295400
Time from diagnosis to HSCT	-6.59594	-1.13894	0.273844
Number of transplanted MNC	0.46029	1.75179	0.101675
Number of transplanted CD34+ cells	0.16634	0.77478	0.451358
Transfusion of Erythrocytes	0.00532	0.02629	0.979399
Transfusion of Platelets (RD)	0.73295	3.93110	0.001507*
Transfusion of Platelets (SD)	-0.13368	-0.68497	0.504544
Transfusion of FFP	0.07233	0.36000	0.724219

\*statistically significant, Plt-platelets, HSCT-hematopoietic stem cell transplantation, MNC-mononuclear cells, RD-random-donor, SD-single-donor, FFP-fresh frozen plasma.

The significance of the coefficient of multiple correlations (R) tested by F-distribution showed that the influence of possible predictive variables on platelet engraftment in autologous transplanted patients with MM is statistically significant for p = 0.004381. The analysis of independent variables showed that transfusion support with random donor platelets had a significant influence on platelet engraftment in autologous transplanted patients with MM (Beta = 0.73295; p = 0.001507). The influence of other predictive factors of interest was not statistically significant in this investigated group (Table 6).

## Discussion

Our study showed that there was not statistically significant difference in the neutrophil and platelet engraftment time between the autologous transplanted patients with AML, lymphoma and MM. On the other side, neutrophil and platelet engraftment occurred more rapidly in patients with multiple myeloma and lymphoma when compared to patients with leukaemia, myelodysplastic syndrome and aplastic anaemia in the study of Goncalves TL et al., suggesting that residual leukaemia in the patient may contribute to delayed engraftment. Other factors, included the drug used in the conditioning regimen, where Cyclophosphamide + BCNU + VP-16, Melphalan and Fludarabine + Cyclophosphamide showed faster engraftment. Age 50–59 years demonstrated faster engraftment. Sex did not influence neutrophil and platelet recovery in this study [7]. Quite the opposite, sex had a significant influence on the neutrophil engraftment in autologous

transplanted patients with AML and lymphoma in our study, and on platelet engraftment in autologous transplanted patients with lymphoma. Factors affecting neutrophil recovery in the not- myeloid malignancy (NNM) group of patients in the study of Carral A et al. were the CD34+ cell number and the CFU-GM dose infused, whereas, for platelet recovery, previous chemotherapy remained significant. In the AML group, hematopoietic recovery was slower than in the NMM group (12 vs. 14 days; p = 0.01). The CD34+ cell dose administered and patient's age were the only factors significantly affecting the speed of neutrophil recovery, while the CD34+ cell dose significantly influenced platelet recovery in these patients. In the NMM group, the most discriminating cut-off values for a rapid neutrophil and platelet recovery were 1.5x10<sup>6</sup> and 2.5x10<sup>6</sup> CD34+ cells/kg, respectively, and for AML patients these figures were 1.5x10<sup>6</sup> and 4x10<sup>6</sup> CD34+ cells/kg, respectively [13]. History of pre-transplant radiotherapy, type of growth factor used for HSC mobilisation and the number of infused CD34+ cells for neutrophil engraftment and history of pre-transplant radiotherapy for platelet engraftment were independent variables that influenced hematopoietic recovery in autologous transplanted patients in the study of Turk HM at al. [20]. Olivieri A et al. [24] found out that the number of transplanted CD34+ cells >5x10<sup>6</sup>/kg in autologous patients is optimal for rapid neutrophil and platelet engraftment. Neutrophil engraftment occurred on day 11 (range 8-15) and platelet engraftment on day 12 (range 8-24). Patients who received 5.0-7.8x10<sup>6</sup>/kg CD34+ cells had shorter neutropenia, needed fewer platelet transfusions and spent less time in the hospital than those who received fewer cells, while patients transplanted with a larger number of cells did not have any advantages. Neutrophil and platelet engraftment was significantly prolonged when transplanted <2x10<sup>6</sup>/kg CD34+ cells in autologous patients (12 vs. 10 days, p = 0.014, respectively 16 vs. 13 days p = 0.0001) in the study of Villalon L et al. [25]. Platelet engraftment was affected with alkylating agents and refractory disease. Neutrophil engraftment was longer in patients with chronic myeloid leukaemia, acute myeloid leukaemia and refractory disease. In patients who received more than >2x10<sup>6</sup>/kg CD34+ cells, Cox model did not identify prognostic factor for hematopoietic recovery. Although mobilising strategy and disease status influenced not only the number of collected HSC but the engraftment kinetics as well, this study showed that the number of infused CD34+ cells is the main predictor of hematopoietic recovery. Possible predictive factors that could influence neutrophil and platelet engraftment: sex, age, diagnosis, number of CD34+ cells infused, time from diagnosis to HSCT, number of apheresis, conditioning, and G-CSF support, investigated, in the study of Ergene U et al. revealed that the number of CD34+ cells >10x10<sup>6</sup>/kg, time from diagnosis to HSCT is longer than a year and conditioning with BEAM were statistically significant for neutrophil engraftment in the univariate analysis. In

the multivariate analysis, none of these factors was significant. The number of CD34+ cells  $>7 \times 10^6/\text{kg}$ , diagnosis of myeloma multiplex, time from diagnosis to HSCT, beginning of G-CSF ( $>2$  days) were statistically significant in the univariate analysis for platelet engraftment, while in the multivariate analysis only the number of CD34+ cells was significant [26]. Similarly, the number of transplanted MNC and CD34+ cells significantly influenced neutrophil recovery in autologous transplanted patients with AML in our study. Mobilisation strategy significantly influenced neutrophil recovery in autologous transplanted patients with lymphoma in our study, while age, time from diagnosis to harvesting and time from diagnosis to HSCT significantly influenced platelet recovery in the same group of patients. On the contrary, another study [27] showed that larger number of infused CD34+ cells and fewer days for collection of HSC (minimum  $2 \times 10^6/\text{kg}$  CD34+ cells) did not provide faster engraftment. Thus mobilisation and harvesting of HSC should not be used as independent factors to predict engraftment. In the study of Alshemmari SH et al. [28], the number of autologous transplanted CD34+ cells was the most significant parameter of platelet engraftment. Furthermore, the number of transplanted CD34+ cells did not significantly affect neutrophil engraftment. The median number of transplanted CD34+ was  $7 \times 10^6/\text{kg}$  (0.38-15), neutrophil engraftment occurred on day 12 [10-15] and platelet on day 11 [6-33]. Other investigated factors didn't affect the engraftment kinetics of the investigated group. Tricot G et al. pointed out in their study that prudent use of stem cell-damaging agents, such as Melphalan, is recommended in patients with refractory multiple myeloma who might be candidates for autologous transplantation. Authors suggested that peripheral blood stem cells should be collected early after diagnosis. Fast recovery of platelets to greater than  $50 \times 10^9/\text{l}$  within 14 days after high-dose cyclophosphamide and  $\leq 12$  months of prior chemotherapy were the best predictors of early engraftment [29]. Yuan S et al. [30] investigated the post-transplant outcomes of 105 adult patients with lymphoma and compared the post-transplant outcomes of 21 patients who received Plerixafor in addition to G-CSF  $\pm$  chemotherapy, because of poor mobilisation, with those of 84 patients who mobilised well without Plerixafor. Despite collecting significantly lower CD34+ cell doses (median of 3.41 vs.  $6.05 \times 10^6/\text{kg}$ ,  $p < 0.0001$ ) than control patients and requiring more collection days, Plerixafor-mobilized patients showed comparable early engraftment characteristics except for slightly delayed neutrophil engraftment (median 11 vs. 10 days,  $p = 0.002$ ) and lower median neutrophil counts (2.1 vs.  $2.6 \times 10^9/\text{l}$ ,  $p = 0.04$ ) at one month after transplant. The most important factor influencing platelet recovery of autologous transplanted patients with multiple myeloma and lymphoma was a diagnosis, followed by the amount of reinfused CD34+ cells. Blood group O+

had the fastest platelet recovery, whereas blood group A harvested the highest cell amounts in the study of Ungerstedt JS et al. [31]. Recipients of transplanted hematopoietic stem cells often require a significant amount of transfusions, especially the ones with delayed hematopoietic recovery and platelet alloimmunization. Evaluation of clinical factors affecting transplant engraftment and transfusion utilisation in the study of Liesfeld J et al. found out that graft type, donor type and the conditioning regimen intensity significantly affected both the neutrophil engraftment time and the platelet engraftment time. Transplanted patients required an average of 6.2 red cell units, and 7.9 platelet transfusions in the first 100 days. Female gender, unrelated donor transplant, leukaemia, high conditioning regimen intensity and receiving TBI were associated with the higher need for RBC unit transfusion. The number of transfusions administered was not affected by age, CD34+ cell number infused or graft type. Among autologous transplanted patients, 5% required neither red blood cells, nor platelet transfusions, as well as some patients who received reduced-intensity conditioning [32]. Among the autologous transplanted patients in our study, there was only one patient (1.1%) who didn't receive any transfusion. The biggest consumers of erythrocyte transfusions in our study were lymphoma patients (83.3%), with average of 3.5 units (range 2-12), The biggest consumers of platelet transfusions were AML patients (100%) with average of 35.1 random-donor platelet units equal to  $\sim 7$  adult therapeutic doses [22], and the biggest consumers of FFP were lymphoma patients (100%) with average of 5.6 units till hematopoietic recovery. MM patients received fewer transfusions than the other two subgroups. The need for red cell and platelet transfusion may vary significantly depending on the type of transplantation and underlying disease. There were 30 (11.6%) patients who did not require platelet support, 52 (20.0%) did not require RBC support and 13 (5.0%) had no transfusions of either RBCs or platelets in the investigated group of 259 autologous transplanted patients, in the study of Gamguly S et al. [33]. Transfusion of blood products is an expensive but integral part of HSCT.

Random donor platelet transfusion was one of the factors that had the biggest influence on the neutrophil recovery in autologous transplanted patients with AML, in our study, as well as the platelet recovery in autologous transplanted patients with AML and MM, which implies that transfusion support with blood component is essential for effective HSCT and fast hematopoietic recovery.

Rapid and durable hematopoietic recovery following HSCT is a prerequisite for successful hematopoietic stem cell transplantation. Additional studies are necessary to better understanding of engraftment kinetic to minimise the use of transfusions and potential complications related to HSCT, as well as to reduce the expenses associated

to HSCT and improve the safety of hematopoietic stem cell transplantation.

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