NEUTROPHIL ENGRAFTMENT AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PEDIATRIC NON-MALIGNANT HEMATOLOGICAL DISORDERS

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Abstract

Objective: To evaluate the neutrophil engraftment after Hematopoietic Stem Cell Transplantation (HSCT) in a group of pediatric patients diagnosed with non-malignant hematological disorders and to relate the possible effects of different patients' and transplant variables such as gender, age, conditioning protocol, graft vs host disease (GVHD) etc., on the neutrophils engraftment.

Methods: 17 children who had non-malignant hematological disorders and who underwent allogeneic HSCT using peripheral blood stem cells (PBSC) as stem cell source were included. Neutrophils engraftment was defined as the first of three consecutive days in which the peripheral blood absolute neutrophilic count was ≥ 500/mm3.

Results: Neutrophils engraftment occurred at a mean of 13.41 ± 3.83 days post-transplant. There was no relation between neutrophil engraftment and gender, original disease, cytomegalovirus (CMV) reactivation, acute GVHD, chronic GVHD, conditioning protocol and GVHD prophylaxis protocol. There were no correlation between the date of neutrophil engraftment post HSCT and age of patients at HSCT, age of donors at HSCT and CD34 count of infused stem cells.

Conclusion: When using PBSC as stem cells source, neutrophils engraftment occurred at a mean of 13.41 ± 3.83 days post-transplant, faster than reported with other stem cell sources. The neutrophils engraftment appears not to be affected with possible effects of different patients' or other transplant variables.

Keywords: Hematopoietic stem cell transplantation - Immune reconstitution - non-malignant hematological disorders – Neutrophils engraftment

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1. INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is now widely used for the treatment of children with blood diseases. An important factor in the prognosis of the patient post-transplant is host immune reconstitution (1). One of the aspects of immune reconstitution is the neutrophil engraftment, which is an important first line of defence against infection (2).

Numerous studies on neutrophil engraftment post-SCT in adults have been reported (3). These studies reported that neutrophil engraftment occur in the first 2 weeks to 1 month post-transplant according to stem cells source, being delayed in umbilical cord blood (UCB) compared to bone marrow (BM) and PBSC stem cells source (1,3,4,5).

Besides stem cell source, factors reported in adults that affect the neutrophil engraftment include human leucocyte antigen (HLA) mismatch between donors and recipients (6,7), CD34+ dose (3,5,8,9), use of ATG (10,11) and conditioning with total body irradiation (TBI) in cord blood transplantation (CBT) (12).

Studies on neutrophil engraftment in pediatric population are few (13,14,15,16,17). Herein we report a study of neutrophil engraftment and factors affecting it in a group of pediatric patient undergoing full matched related HLA HSCT for non-malignant

hematological disorders, using PBSC as stem cell source.

2. PATIENTS AND METHODS

1. Patients cohort

This observational cohort study included 17 pediatric patients (age less than 18 years old) diagnosed with non-malignant hematological disorders based on conventional clinical and hematologic criteria. The patients underwent HLA fully matched allogeneic HSCT at the Pediatric Hematology and Bone Marrow Transplantation Centre, Cairo University from December 2015 to December 2019. The study included 12 patients with bone marrow failure and 5 patients with beta thalassemia major. Patients' characteristics are illustrated in table 1.

2. Cohort transplant methods

Patients' characteristics are illustrated in table 1. Regarding conditioning regimen, the BU+CY regimen consisted of a myeloablative regimen in the form of oral busulfan 20mg\kg total dose and intravenous cyclophosphamide 200mg\kg total dose. The FLU+CY regimen was a reduced intensity regimen in the form of intravenous Fludarabine 120mg\m2 total dose and intravenous cyclophosphamide 200mg\kg total dose. Regarding GVHD prophylaxis, the MTX based regimen given to bone marrow failure group (12 patients) consisted of 2 or 3 doses (according to occurrence of side effects) of methotrexate with initial dose of 15mg\m2 and subsequent doses of 10mg\m2. The ATG based regimen given to thalassemia major patients consisted of intravenous antithymocyte globulin (ATG) 11mg\kg\day for 3 days pre transplant and 3 days post-transplant. In addition, all patients in either group received initial cyclosporine starting from D-1. Source of stem cells in all patients was G-CSF mobilized PBSC. G-CSF where given to the donor on a dose of 10 microgram\kg\day starting from D-5 till D-1.

Neutrophils engraftment: defined as the first of 3rd consecutive days in which the peripheral blood absolute neutrophilic count was ≥ 500 (18).

For assessing CMV reactivation post transplant, CMV PCR was performed twice weekly to all patients starting from D+14. CMV reactivation was defined as > 1000 copies\ml.

Grading of acute graft versus host disease (GVHD) was done according to Gluksberg criteria (20).

3. STATISTICAL ANALYSIS

All data were collected, tabulated and statistically analyzed using SPSS 26.0 for windows (SPSS Inc., Chicago, IL, USA). Quantitative data were expressed as the mean ± SD & median (interquartile range), and qualitative data were expressed as absolute frequencies (number) & relative frequencies (percentage). Mannwhitney test was used to compare between more than two groups with non normally distributed variables. Percent of categorical variables were compared using Chi-square test. All tests were two sided. P-value < 0.05 was considered statistically significant (S), p-value $\geq$ 0.05 was considered statistically insignificant (NS).

3. RESULTS

Post-transplant data is revealed in **Table (1).** Neutrophils engraftment occurred at a mean of 13.41 ± 3.83 days post-transplant. Four (23.5%) patients (2 aplastic anemia patients, one patient with congenital neutropenic, and one patient with beta thalassemia major) were given G-CSF to aid in neutrophil engraftment.

Table (1): Patients characteristics and post transplant data

Donor age at time of HSCT (yrs) (Mean ±SD)	18.09 (±12.78) (Range 3.1-40.1)
Patient age at time of HSCT (yrs) (Mean ±SD)	5.59 (±3.36) (Range 0.6-14.6)
Patient gender (No. of patients (%)) • Male • Female	• 10 (58.8 %) • 7 (41.2%)
Patients' diagnoses (No. of patients (%)) • Severe aplastic anemia • Beta thalassemia major • Congenital neutropenia • Osteopetrosis	• 10 (58.8%) • 5 (29.4%) • 1 (5.9%) • 1 (5.9%)
Conditioning (No. of patients (%)) • Fludarabine + Cyclophosphamide (FLU+CY)	• 11 (64.7%) o *10 SAA patients (58.8%)* o *1 Congenital neutropenia (5.9%)*

• Busulfan + Cyclophosphamide (BU+CY)	• 6 (35.3%) ○ *5 beta thalassemia (29.4%)* ○ *1 osteopetrosis (5.9%)*
CD34+ count (x10^6\kg) (Mean ±SD)	10.56 (±2.6)
Neutrophils engraftment (Mean ±SD)	13.41 ± 3.83
GVHD prophylaxis (No. of patients (%)) • Methotrexate + Cyclosporin	• 12 (70.6%) ○ *10 SAA patients (58.8%)* ○ *1 Congenital neutropenia (5.9%)* ○ *1 osteopetrosis (5.9%)*
• ATG + Cyclosporin	• 5 (29.4%) ○ *5 beta thalassemia (29.4%)*
CMV reactivation (No. of patients (%))	7 (41.2%)
Acute GVHD (No. of patients (%))	• 5 (29.4%) ○ *3 SAA (17.65%)* ○ *2 beta thalassemia (11.75%)*
Chronic GVHD (No. of patients (%))	• 3 (17.65%) ○ *1 SAA (5.90%)* ○ *2 Beta thalassemia (11.75%)*

There was no relation of neutrophil engraftment with gender (p=0.47), initial diagnosis (p=0.28), conditioning protocol (p=0.48), GVHD prophylaxis protocol (p=0.28), CMV reactivation (p=0.53), acute GVHD (p=0.15) or chronic GVHD (p=0.38). **Table (2)**

Table (2): relations some transplant variables to neutrophil engraftment

Variable	Value (mean ± SD)	P value
Gender		
Male	14 ± 4.69	0.47
Female	12 ± 2.14	
Diagnosis		
Bone marrow failure	12.75 ± 2.37	0.28
Beta thalassemia major	15 ± 6.20	
Conditioning		
BU+CY	14.33 ± 5.78	0.48
Flu+CY	12.9 ± 2.42	
GVHD prophylaxis type		
MTX+CSA	12.75 ± 2.37	0.28
ATG+ CSA	15 ± 6.20	
CMV reactivation		
No	12.9 ± 2.37	0.53
Yes	14.14 ± 5.42	
Acute GVHD		
No	14.25 ± 4.08	0.15
Yes	11.4 ± 1.01	
Chronic GVHD		
No	13.78 ± 3.96	0.38
Yes	11.66 ± 0.94	

There was no correlation between neutrophil engraftment and CD34+ cell dose (p=0.42), age of patients at the time of transplant (p=0.96) or age of donor at the time of transplant (p=0.44) **Table (3).**

Table (3): Correlations of some transplant variables to neutrophil engraftment

Correlations	**P value**
Age at BMT	0.959
Donor age at BMT	0.443
CD34 (x106\kg)	0.421

4. DISCUSSION

Our study included 17 patients diagnosed with non-malignant diseases who underwent HSCT.

In our study, PBSC was used as stem cell source. Our study showed that neutrophils engraftment occurred at a mean of 13.41 ± 3.83 days post-transplant. This is in line with other studies in adults or pediatrics using PBSC (4,14) or BMT as stem cell source (14,15), and shorter than studies using cord blood (CB) as stem cells source (13,14,15,16,21). The delayed engraftment in studies using CB as stem cell source is due to the limited numbers of progenitor cells in the UCB unit (21).

Our study didn't show a correlation between neutrophil engraftment and CD34+ cell dose. Other studies in adults showed a correlation between higher CD34+ dose and faster neutrophil engraftment, with different cut off cell dose between ≥ 1.5 x106\kg and ≥ 5 x106\kg associated with faster neutrophil engraftment according to stem cell source (5,8,9,22,23). The reason that our study didn't show a correlation between neutrophil engraftment and CD34+ cell dose maybe due to the fact that all our patients received CD34+ stem cell dose of ≥ 5 x106\kg, as our patients' mean CD34+ dose was 10.56 (±2.6) x106\kg with minimum dose of 7.5 x106\kg and maximum dose of 15 x106\kg.

Our study didn't show a relationship between intensity of conditioning and neutrophil engraftment. This is in line with studies in adults using PBSC or BM as stem cell source as (12,24). Studies on CBT showed enhanced neutrophil engraftment using TBI specially in the settings of more HLA mismatch between donor and recipient (12). This effect may be due to the effect of more intense conditioning of TBI in the settings of HLA mismatch leading to more suppression of recipient immunity which in turn enhances engraftment (12).

Our study didn't show a relation between the use of ATG and neutrophil engraftment. Some studies in adults showed that the use of ATG was associated with delayed neutrophil engraftment (10,25), while other studies both in adults and pediatrics showed no effect (13,26). This discrepancy may be due to the difference in the method of ATG preparation and/or the ATG dose, and the presence of HLA-mismatching in the other studies (10).

Our study didn't show a relationship between gender and neutrophil engraftment. This is in line with other studies performed in adults (27).

Our study didn't show a relation between donor age and neutrophil engraftment. This is in contrast to a study done by Capellie B et al (28) that showed enhanced neutrophil engraftment in sickle cell disease (SCD) patients aged 0-15 years undergoing HLA matched HSCT using BM as stem cell source from sibling donors who were above 9.6 years old compared to younger donors. However the author of the same paper pointed out that this effect maybe due to the fact that the younger in age and thus in weight patient, have received a higher total nucleated cells (TNC) from the older donor compared to the younger donor and not due to true relation to donor's age. (28).

In our study, no relation was found between acute GVHD and neutrophil engraftment. One study reached the same conclusion in patient developing acute GVHD and taking steroids therapy, but showed delayed neutrophil engraftment in patient developing acute GVHD who wasn't on steroids at the time of engraftment (29). This may explain why no relation was found between acute GVHD and neutrophil engraftment, due to the rapid institution of systemic steroids therapy in our patients developing acute GVHD.

This work is among only few studying neutrophil engraftment and factors affecting it in post HSCT pediatric patients due to non-malignant diseases. The relatively small number of patients is the main limitation of this study.

5. CONCLUSION

When using PBSC as stem cells source, neutrophils engraftment occurred at a mean of 13.41 ± 3.83 days post-transplant, faster than reported with other stem cell sources. The neutrophils engraftment appears not to be affected with possible effects of different patients' or other transplant variables.

6. DECLARATIONS

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Declarations of interest: none

Conflicts of interest/Competing interests: None of the authors have any conflict of interest to declare.

Ethics approval: The study was conducted in accordance with the declaration of Helsinki for studies involving human participants and was approved by the institutional research ethics committees at Faculty of Medicine, Cairo University (Approval code: D-21-2019).

Consent to participate: Written informed consent was obtained from the parents. Participant data has been anonymized.

Consent for publication: Written informed consent was obtained from the parents. Participant data have been anonymized.

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