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ORIGINAL ARTICLE

Impact of CD34+ Stem Cell Dose on Engraftment Period in Allogeneic Peripheral Blood Stem Cell Transplanted Patients

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ABSTRAK

Sel stem progenitor hematopoietik yang diperolehi dari darah periferal telah lama digunakan untuk merawat pesakit malignansi hematopoietik. Kejayaan transplantasi allogenik sel stem darah periferal (PBSCT) yang mengambil kira kadar pemulihan hematopoietik sel dan engrafmen adalah bergantung pada jumlah sel stem CD34+ yang disuntik. Kajian ini bertujuan untuk mengaitkan jumlah sel CD34+ yang disuntik dengan tempoh engrafmen. Kajian retrospektif ini melibatkan 62 pesakit dengan malignansi hematopoietik yang telah menerima PBSCT di Pusat Perubatan Universiti Kebangsaan Malaysia dari tahun 2011 hingga 2015. Kesan infusi sel stem CD34+ pada engrafmen oleh neutrofil dan platelet dan pencapaian chimerism lengkap telah dikaji. Pesakit dibahagikan kepada dua kumpulan berdasarkan jumlah sel stem CD34+ yang disuntik. Kumpulan A terdiri daripada 9 pesakit yang menerima infusi <5x10⁶ sel/kg CD34+ sel stem sementara kumpulan B terdiri dari 53 pesakit yang disuntik dengan ≥5x10⁶ sel/kg CD34+ sel stem. Data dikumpulkan dan dianalisa. Pesakit Kumpulan B dikaitkan dengan engrafmen neutrofil yang lebih cepat: 12 (10-14) hari sementara kumpulan A adalah 15 (10-21) hari (p=0.002). Engrafmen platelet juga lebih cepat dalam kumpulan-B: 17 (12-25) hari berbanding dengan kumpulan A: 18 (15-30) hari. Kimerisme lengkap dalam kumpulan B berlaku dalam 30 (15-90) hari dan pada kumpulan A adalah 60 (30-240) hari. Kadar pencapaian engrafmen platelet (p=0.149) dan kimerisme lengkap (p=0.021) tidak banyak dipengaruhi oleh jumlah sel CD34+. Kajian ini telah menunjukkan bahawa infusi sel stem ≥5x10⁶ sel/kg CD34+ sel induk dapat memendekkan masa untuk engrafmen hematopoietik sel terutamanya engrafmen

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neutrofil.

Kata kunci: engrafmen, jumlah sel stem CD34⁺, kimerisme, transplantasi allogenik

ABSTRACT

Haematopoietic progenitor stem cells acquired from the peripheral blood have been increasingly used to treat patient with haematological malignancy. The success of the allogeneic peripheral blood stem cell transplantation (PBSCT) is significantly dependent on amount of $CD34^+$ stem cell infused which will determine the rate of the haematopoietic recovery and engraftment. This study was aimed to correlate the amount of $CD34^+$ cell infused with the period of engraftment. This retrospective study was conducted on 62 patients with haematopoietic malignancy who have received PBSCT at Universiti Kebangsaan Malaysia Medical Centre from year 2011 to 2015. The impact of $CD34^+$ stem cell infusion on neutrophil and platelet engraftment and obtaining complete chimerism was studied. Patients were divided into two groups based on the amount of $CD34^+$ stem cell. Group A consisted of 9 patients and infused with $<5x10^6$ cells/kg $CD34^+$ cell while Group B consisted of 53 patients and infused with $\geq 5x10^6$ cells/kg $CD34^+$ stem cell. Data were collected and analysed. Group B patients were significantly associated with faster neutrophil engraftment: 12 (10-14) days while group A were 15 (10-21) days, ($p=0.002$). Platelet engraftment was also faster in group B: 17(12-25) days compared to group A: 18 (15-30) days. Complete chimerism in group B occurred in 30 (15-90) days and in group A was 60 (30-240) days. Platelet engraftment ($p=0.149$) and complete chimerism ($p=0.021$) were not significantly influenced by the $CD34^+$ cell count. This study has shown that infusion of $\geq 5x10^6$ cells/kg $CD34^+$ stem cell shorten the time to haematopoietic engraftment particularly of neutrophilic engraftment.

Keywords: allogeneic transplantation, chimerism, engraftment, $CD34^+$ stem cell count

INTRODUCTION

Over the years haematopoietic progenitor stem cells from the peripheral blood have increasingly been used for stem cells transplantation primarily to treat patients with different types of malignant haematologic disorders (Körbling & Freireich 2011). Among the common complications of allogeneic stem cell transplant, graft-versus-host disease (GVHD) is one of the serious or fatal complication where the transplanted donor T-lymphocytes react to foreign host cells and cause injury to the host tissue (Bittencourt et al. 2002; Singhal et al. 2000). Therefore, for every haematopoietic stem cell transplant cases, the best matched Human Leukocyte Antigen

(HLA) donor is identified (Nor Rafeah et al. 2017) to prevent or minimise the risk of GVHD and thus promote engraftment (Cutler & Antin 2001).

Engraftment is a significant milestone in allogeneic stem cell transplant recovery. During engraftment the transplanted stem cells grows and forms new haematological cells and this usually occurs between 14 to 28 days after the allogenic bone marrow transplant (BMT) which is achieved earlier in case of peripheral blood stem cells transplant (PBSCT). In PBSCT, neutrophil engraftment is achieved one to six days earlier while platelet engraftment is achieved four to seven days earlier than BMT (Cutler & Antin 2001). Engraftment is defined as the recovery of neutrophil and platelet after a period of aplasia (Brown 2017). It is considered when the absolute neutrophil count (ANC) is 0.5 x 10^9/L for three consecutive days. The second sign of engraftment is when the platelet count continues to increase ≥20 x 10^9/L for 3 consecutive days without a platelet transfusion in the 7 preceding days (Teltschik et al. 2016). It is important for the clinician to closely monitor the neutrophil and platelet count increment post hematopoietic stem cell transplantation (HSCT) to evaluate engraftment. Another method of monitoring engraftment is by monitoring chimerism in the transplanted patients. In the post transplant patients, determination of the degree of chimeras reflects whether the engraftment is successful or not (Hamidah et al. 2011). Generally chimerism describes the presence of cell populations that were derived from donors of identical or different species occurring spontaneously or produced artificially. Thus the co-existence of cells from two different organisms (evolved from two different zygotes) in one body is called chimerism (Khan et al. 2004). In a number of studies, molecular monitoring of $CD34^+$ stem cell chimerism has been analysed starting at four week post-transplant (Khan et al. 2004; Stahl et al. 2015).

Post allogeneic blood stem cell transplantation results in simultaneous development of both host and donor haematopoietic system for a temporary period referred to as mixed chimerism. However, in successful engraftment, donors cells predominates and when all the cell lineages converted to donor origin, then it is called as complete chimerism. Different techniques are available for the detection of chimerism such as cytogenetics, red cell phenotyping, restriction fragment length polymorphism analysis (RFLP) and fluorescence in situ hybridisation of sex chromosomes. However, all these methods are time consuming and not suitable for every patient (Bader et al. 2005). Polymerase chain reaction (PCR) technique has more advantages. It is performed by amplification of variable number of tandem repeats (VNTR) and in later decades by short tandem repeats (STR). Currently, real-time PCR (rPCR) approaches using single nucleotide polymorphism (SNP) for the detection of chimerism are available. Thiede et al. (2001) suggested that in post allogenic transplant patients during the engraftment period with mixed macro-chimerism i.e. chimerism between

3 and 97%, STR analysis using a multiplex assay should be used on whole blood or bone marrow samples to quantify chimerism. The authors also suggested that rPCR should be used for further monitoring process once the chimerism in the peripheral blood has reached a level greater than 97%.

Clinical studies have shown that the success of the allogeneic transplant and the rate of engraftment is significantly dependent on the amount of $CD34^+$ stem cell in the donor materials (Bai et al. 2014; Bittencourt et al. 2002; Yamamato et al. 2018; Remberger et al 2015). In many centres, the recommended dose of the $CD34^+$ stem cell count is between 2-5 x 10^6 $CD34^+$ cells/kg as this ensure rapid engraftment and lower the risk of graft failure. Nevertheless, $CD34^+$ stem cell count of 1-2 x 10^6 $CD34^+$ cells/kg is acceptable (Yamamoto et al. 2018). It is also established that infusion of a stem cell dose of more than 5 x 10^6 $CD34^+$ per kg increased the probability of rapid durable engraftment, improved overall survival (OS), and a lower incidence of transplantation-related mortality (TRM), however increases incidence and severity of chronic GVHD (cGVHD) (Yamamoto et al. 2018)).

It is important to evaluate the impact of $CD34^+$ dose and the engraftment. Our study was aimed to evaluate the impact of $CD34^+$ stem cell infusion with the period of neutrophil and platelet engraftment and period to achieved chimerism in patients who have undergone allogeneic HSCT in our Centre, Universiti Kebangsaan Malaysia Medical Centre (UKMMC).

MATERIALS AND METHODS

Study Design

This retrospective study included all 62 patients with haematological malignancy who have received allogeneic PBSCT in UKMMC, from the year 2011 to 2015. The impact of $CD34^+$ stem cell infusion on neutrophil and platelet engraftment and obtaining complete chimerism was studied. Ethical approval was obtained from the Research Ethics Committee of UKMMC and the study was conducted in the Stem Cell Transplantation Unit and Molecular Unit, UKMMC.

Patients

The patients were divided into two different groups based on the amounts of $CD34^+$ stem cell infusion. Group A consisted of nine patients who were infused with <5 x 10^6 cells/kg $CD34^+$ stem cell. Group B consisted of 53 patients, who were infused with $CD34^+$ stem cell count ≥5 x 10^6 cells/kg. In both the groups, the number of days were determined when patients achieved complete chimerism and neutrophil and platelet engraftment occured.

Clinical engraftment: Neutrophil engraftment was considered when the absolute neutrophil count (ANC) was achieved ≥0.5 x 10^9/L for three consecutive days. Platelet engraftment was considered when the platelet count continues to increase more than ≥50 x 10^9/L for three consecutive days.

Data Collection

This retrospective study involved data collection from patients with haematopoietic malignancy who had undergone allogeneic PBSCT. Data of the amount of CD34+ stem cell infusion during allogeneic PBSCT and days at which patients achieved complete chimerism were retrieved from protocol files and patients records that were stored in the Stem Cell Transplantation and Molecular Laboratory. While the neutrophil and platelet count data were retrieved from the Integrated Laboratory Management System (ILMS) of UKMMC.

Statistical Analysis

All the results obtained were analysed using Statistical Package for the Social Sciences (SPSS) software version 21.0 (IBM Corp., Armonk, NY, USA). Data on time of neutrophil and platelet engraftment and complete chimerism were expressed as median and range. Non-parametric Mann-Whitney U test was used for the comparison between two groups of patients based on the total CD34+ stem cell infusion. Normality test was assessed using Shapiro-Wilk test and found data were not normally distributed and thereby proceeded with the non-parametric test. Kaplan-Meier statistical analysis was used to evaluate the difference in engraftment of neutrophil and platelet as well as complete chimerism between the two groups. The results obtained were compared using the log-rank test in order to determine statistical differences. Differences with probability of $p<0.05$ were accepted as statistically significant.

Table 1: Demographic data of the patient (N=62)

Variables		Number	P value
Gender	Female	30	0.716
	Male	32	
Age	Median years (range)	32.5 (15-61)	0.001
Diagnosis	AML	28	
	ALL	13	
	CML	7	
	MDS	7	
	Lymphoma	2	
	Aplastic anaemia	5	
CD34+ stem cell count (cells/kg); Median (range)		7.2 (1.01-12)	0.241
Days of complete chimerism	Mean (range)	30 (15-240)	0.975
CD34+ cell infused in Group A (cells/kg) (n=9)	Median range	4.1 1.01-4.92	0.002
CD34+ cell infused in Group B (cells/kg) (n=53)	Median range	7.4 5.2-12	

Table 2: Correlation of CD34+ stem cell counts with outcome (N= 62)

Variables	Group A	Group B	Log rank	P value
Neutrophil engraftment (days)	15 (10-21)	12 (10-14)	0.001	0.002
Platelet engraftment (days)	18 (15-30)	17 (12-25)	0.175	0.149
Complete chimerism (days)	60 (30-240)	30 (15-90)	0.021	0.021

RESULTS

The patients consisted of those aged between 15 to 61 years. The median age of the transplant patients was 32.5 years (15-61). The allogeneic stem cell transplants were done on total 62 patients where 32 were male patients and 30 were female patients. Among these patients, 28 patients were diagnosed with acute myeloid leukaemia (AML), 14 patients with acute lymphoblastic leukaemia (ALL), 7 patients with myelodysplastic syndrome (MDS), 7 patients with chronic myeloid leukaemia (CML), 2 patients with lymphoma and 5 patients with aplastic anaemia. The median for CD34+ stem cell count that were infused to the patients were 7.1 x 10^6 (1.01-12 x10^6) cells/kg. The CD34+ stem cell count was the predictor variables during allogeneic PBSCT. The median days to achieve complete chimerism was 30 days (range 15-240 days). The primary outcome was the time to neutrophil engraftment and platelet engraftment above their threshold value for three consecutive days. The secondary outcome was the period to achieve complete chimerism starting from the first day of post-transplant.

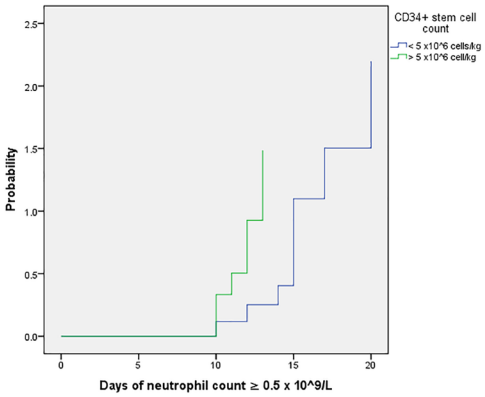

Figure 1: Kaplan-Meier cumulative probability curves demonstrating the time to neutrophil engraftment to ≥0.5x10^9/L versus CD34+ stem cell count infuse to allogeneic stem cell transplant patients.

Median (range) for the infused CD34+ stem cell count in Group A was 4.1 (1.01-4.92) cells/kg while for Group B was 7.4 (5.2-12) cells/kg and the difference were statistically significant (p=0.002) (Table 1).

The impact of CD34+ stem cell count on the rate of neutrophil and platelet engraftment and achievement of complete chimerism for all post PBSCT patients were presented in Table 2. The time taken for Group B to achieve complete chimerism after transplant was significantly faster than Group A (p value=0.021). Similarly, for neutrophil engraftment, group B showed significantly faster engraftment than group A (p value=0.002). Although platelet engraftment in group B was faster, it did not show any statistically significant increment than group A (p value=0.149). Figure 1, 2 and 3 shows the Kaplan-Meier cumulative

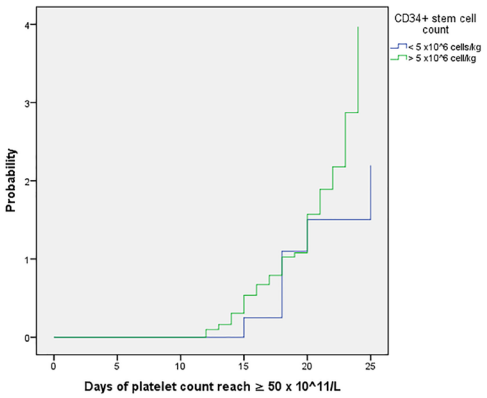

Figure 2: Kaplan-Meier cumulative probability curves demonstrating the time to platelet engraftment to ≥50x10^{11}/L versus CD34^{+} stem cell count infuse to allogeneic stem cell transplant patients.

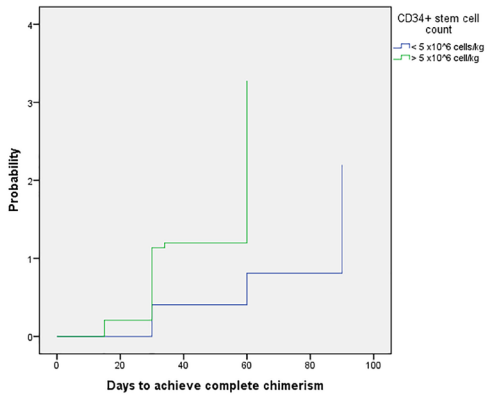

Figure 3: Kaplan-Meier cumulative probability curves demonstrating the time to achieve complete chimerism versus CD34^{+} stem cell count infuse to allogeneic stem cell transplant patients.

probability curves demonstrating the difference in time of engraftment of neutrophil and platelet as well as complete chimerism. Figure 1 and 3 again reiterate the significant rate to achieving neutrophils engraftment and complete chimerism respectively by Group B. Figure 2 demonstrates Group A showing a steady but delayed increment of platelet count and thus engraftment of the platelet as compared to Group B.

DISCUSSION

The use of PBSCT has been extensively used in the treatment of several haematological malignancies (Nivison-Smith et al. 2016). The rate of haematopoietic recovery depends on the rate of stem cell engraftment which partly depends on the CD34^{+} stem cell count that has been transplanted.

We examined the correlation between neutrophil engraftment, platelet engraftment and the days to achieve complete chimerism with the CD34^{+} stem cell count. We observed a faster and steady rise in the neutrophil and platelet counts following the allogeneic PBSCT and thus in the rate of complete chimerism achieved when the number of CD34^{+} stem cell count infused was ≥5 x 10^{6} cells/kg. It is of utmost importance to define the amount of CD34^{+} cell and infuse an appropriate number of CD34^{+} cells to secure engraftment and to avoid infusion of excess CD34^{+} cells to minimise the risk of chronic GVHD (Kamel et al. 2005).

Generally, the period to achieve engraftment is faster when patients are infused with higher dose of CD34^{+} stem cell count (Nakamura et al. 2008; Kamel et al. 2005; Bolwell et al. 2007; Kakihana et al. 2010). The number of CD34^{+} stem cells required to achieve consistent engraftment is mentioned as 2-5 x 10^{6} cells/kg of body weight (Lemos et al. 2018). These findings were concurred by another more elaborate

study by Ashihara et al. (2002) where patients were divided into three groups based on $CD34^+$ cells : <2.5 x 10^6/kg (group A), ≥2.5 to 5 x 10^6/kg (group B) and ≥5 x 10^6/kg (group C). The median time for haematopoietic recovery for neutrophil was 9 days in group C which was significantly shorter compared to 11 days for both group A and group B. For platelet engraftment, median time was 14 days in group C compared to 17 and 18 days in group B and A, respectively (Ashihara et al. 2002). Similarly, in another study, neutrophil engraftment was affected by the $CD34^+$ cell amount, where 5.0-7.8 x 10^6 $CD34^+$ cells/kg allowed a significantly faster engraftment than <2.5 x 10^6 $CD34^+$ cells/kg (p=0.0312) (Olivieri et al. 1998). Another recent study comparing the standard group (2 to 5 x 10^6 cells/kg), low group (1 to 2 x 10^6 cells/kg), and very low group (<1 x 10^6 cells/kg) showed the median time to neutrophil engraftment was 14 days, 15 days, and 15.5 days, respectively. While the median time to platelet engraftment was 20 days, 27 days, and 35 days, respectively. Thus, higher cell count gives early recovery. However, $CD34^+$ cell count of 1 to 2 x 10^6 cells/kg gives an acceptable results (Yamamoto et al. 2018). Our study had also demonstrated that patients receiving ≥5 x 10^6 cells/kg body weight took shorter time to achieve engraftment compared to patients receiving <5 x 10^6 cells/kg body weight of $CD34^+$ stem cell count.

Although in our study, higher stem cell count gives an earlier recovery, only the neutrophil engraftment had correlated well with the higher dose of $CD34^+$ stem cell count (p=0.002). Although platelet engraftment was faster with a higher dose of $CD34^+$ cells, nevertheless there was no clear statistical significance (p=0.149) observed. Some studies have also demonstrated that the $CD34^+$ stem cell count did not affect the rate of platelet engraftment after PBSCT. A study by Kamel et al. (2005) also showed no significant correlation to platelet engraftment while neutrophil engraftment showed significant correlation to total $CD34^+$ cell dose. Study by Nakamura et al. (2008) also found a significant relationship between higher $CD34^+$ cell dose and faster neutrophil engraftment. However, there was no statistical significance for platelet engraftment. The engraftment rates were also affected by many other factors such as graft source, different conditioning regimens and forms of chemotherapy used prior to the stem cell transplant. Furthermore, diverse patient population with different types of cancer and severity may also affect the engraftment rate (Hutt 2018; Remberger et al. 2015). Nevertheless, we acknowledge the need for bigger sample size in order to determine if true correlation exists.

Regarding days to achieve complete chimerism, this present study showed that the amount of $CD34^+$ stem cell count was significantly associated with the achievement of early complete chimerism. Group B patients who had received ≥5 x 10^6 cells/kg $CD34^+$ stem cell were able to achieve full chimerism (15-90 days) faster than Group A patients (30-240 days) who had received ≤5x10^6 cells/kg.

In the past, several studies had also shown similar results with complete chimerism achieved by day +30 when infused with $CD34^+$ stem cell count greater than $5x10^6$ cells/kg (Bader et al. 2005; Bornhauser et al. 2009). Therefore, optimisation of $CD34^+$ stem cell count may improve the days to achieve complete chimerism. Previous study by (Holtan et al. 2010) suggested that patients who were infused with higher dose of $CD34^+$ stem cell showed rapid chimerism and lower rejection rate post-transplant. Again, we acknowledge that in this present study the sample size was small especially for Group A patients who were infused with <5 x10^6cells/kg $CD34^+$ stem cell. The collection of a larger data set from a bigger sample size, possibly from a pooled multicentre study data would definitely improve the power of the study.

CONCLUSION

Our study showed that $CD34^+$ stem cell count remains a convenient and reliable marker for assessing the rate of engraftment after allogeneic peripheral blood stem cell transfusion. Our results have shown that $CD34^+$ stem cell count $\geq 5 \times 10^6$ cells/kg was significantly associated with rapid and durable neutrophil engraftment as well as complete chimerism; although platelet engraftment is not significantly associated. The influence of a higher $CD34^+$ stem cell count on the overall rate of engraftment is still debatable, especially when taking into account its higher risk of GVHD post-allogeneic transplantation. We recommend extending this study with collection of more data in order to increase the power of this study.

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