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Immature Reticulocyte Fraction in Guiding Stem Cell Harvest in Autologous Peripheral Blood Stem Cell Transplant

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ABSTRACT

Peripheral blood (PB) CD34+ cells enumeration is currently the most reliable method to guide the timing of stem cell harvest. However, its usage is restricted by being technically challenging, costly, and time-consuming. Immature reticulocyte fraction (IRF) determination, which is simpler and cheaper and has a faster turn-around time, has been proposed for a similar purpose. The purpose of this study is to evaluate the value of IRF in guiding stem cell harvest and examine the correlation between IRF and PB CD34+ cells count. Daily pre-harvest tests, i.e. PB CD34+ cells and IRF from 21 patients scheduled for autologous PBSC transplant were assessed. Stem cells harvests were commenced when the PB CD34+ cell count were more than 10 cell/ul. A total of 205 pre-harvest tests were analysed. Following stem cell mobilisations, both the IRF and PB CD 34+ cell counts rose with a variable pattern. In this study, we observed that the IRF peaks preceded the PB CD34+ count by 2 days. On the day of stem cell harvest, all the peak IRF values were >0.3. The PB CD34+ cell counts correlated with the harvested stem cell yield, whereby $r^2 = 0.77$, $p < 0.021$. In autologous stem cell mobilisation, we believe that IRF is a useful screening tool to predict the rise of the PB CD34+ cell counts as it is a simple, fast and less costly. An IRF of > 0.3 may be used as a cut-off value for the initiation of PB CD34+ quantification prior to stem cell harvest.

Keywords: Autologous PBSCT, PB CD34+ count, immature reticulocyte fraction

INTRODUCTION

Autologous peripheral blood stem cell transplant (PBSCT) has been widely used as a treatment tool in various haematological disorders. Stem cell yield of more than 2×10^6/kg body weight recipient is critical to ensure engraftment in PBSCT.[1,2,3] The widely accepted method of using PB CD34+ cells as an indicator for stem cell harvest is restricted by being technically challenging, costly and time-consuming.

The immature reticulocyte fraction (IRF) is a quantification of the youngest reticulocyte population and is expressed as a fraction. Its determination by an automated haematology analyser can signal a rebound in haematopoisies.[4] Studies[5,6,13] have shown that IRF was able to predict the timing of stem cell collection. Our study aimed to evaluate the IRF which is a cheaper, simpler and less time consuming test, as an alternative in optimizing the timing for stem cell collection in a local setting.

PATIENTS AND METHODS

This observational study was conducted in Hospital University Kebangsaan Malaysia, and involved patients who were planned for upfront autologous PBSCT. Informed consent for peripheral blood stem cell collection and transplantation in this study was obtained from the patients or their guardians prior to the sample collection.

All patients were treated with combination chemotherapy according to their diseases until they achieved partial or complete remission. These patients were then mobilised with chemotherapy and Granulocyte-Colony Stimulating Factor (G-CSF). One day after completion of the mobilisation chemotherapy, G-CSF 10μg/kg was given twice daily to enhance stem cell mobilizations. In view of our previous observation that the median day of stem cell harvesting using various mobilization chemotherapies and G-CSF protocols was day 14, we, therefore, chose day 10 of chemotherapy to start the monitoring of IRF and peripheral blood CD34+ counts. On day 10 of onward chemotherapy, 3 ml of peripheral blood was collected in a EDTA anti-coagulated specimen tube for the measurement of CD34+ cells count and IRF level. Serial enumeration of PB CD34+ cell count and IRF level were monitored daily until the day of stem cell harvesting or documented failed mobilisation as determined by the transplant physician. To ensure good engraftment in PBSCT, previous authors have reported that the minimum number of CD34+ cells must be at least 2×10^6 CD34+ cells/kg recipient's body weight[2,3]. According to previous studies, a peripheral blood CD34+ cell count of ≥ 10 cells/μl has been used widely as a cut-off value for initiation

of peripheral blood stem cell harvesting[14,15]. In our centre, we also used this PB CD34+ cells count ≥ than 10 cells/μl to initiate the stem cell harvesting.

Peripheral blood stem cell harvesting was performed using a continuous-flow blood cell separator (COBE Spectra). Manual white blood cell (WBC) protocol was used, in which two times the patients' blood volume were processed in each procedure. At the end of the stem cell harvesting procedure, the collected stem cell product was processed and a 1 ml of the stem cell product was obtained under sterile condition for the analysis of CD34+ cell yield. Both the CD34+ cell counts from the pre-harvesting peripheral blood and collected stem cell product were identified using ProCOUNT™ enumeration kit protocol and measured using multi-parameter gating strategy on a fluorescence-activated cell sorter flowcytometer (FACScan Becton Dickinson). The IRF was measured based on fluoro-flow cytometry and semiconductor technologies using the automated haematology analyser Beckman Coulter Gen-S System II.

Spearman Rho in SPSS version 12 was used to observe the correlation between PB CD34+ counts, IRF and CD34+ cell counts of the stem cell yields.

RESULTS

Patients, Cell Counts in PB and Apheresis Product

A total of 21 patients planned for autologous PBSCT were recruited within a 10-month duration of the study period. Their median age was 41 years (range, 14-62 years old) and the ratio of male to female was 3.2:1. There were 11 Malay patients (52.4%), 9 Chinese patients (42.9%) and 1 Indian patient (4.8%). Fifty two percent were diagnosed as relapsed or refractory Non-Hodgkin lymphoma, 19.0% with relapsed Hodgkin's disease and 28.6% with acute leukaemia (Table 1). 66.7% of these 21 patients were able to achieve complete remission prior to stem cell mobiliation.

Following stem cell mobiliation, only 12 out of 21 patients aged between 14 to62 years (median = 23), were successfully mobilised and had undergone stem cell collection. Five patients were considered as poor mobilisers where their CD34+ counts failed to reach the targeted cut-off of ≥ 10 CD34+ cell/μl up to day 17 of chemotherapy and the daily CD34+ counts showed decreasing trends. The reasons for the poor mobiliation could be secondary to the multiple courses of chemotherapy with poor bone marrow reserve in these patients. In 4 other patients, the mobiliation protocol and harvesting procedure could not be continued because two ~~2~~ of them developed complications, i.e. one patient with intracranial bleed and septicaemic shock following mobiliation chemotherapy, while another patient with medical co-morbidity developed acute coronary syndrome during mobiliation chemotherapy and the remaining patients had incomplete data (see Table 1).

Immature Reticulocyte Fraction (IRF)

The patterns of the IRF levels were very variable in these patients. Some patients showed the IRF was zero on day 10 post mobiliation chemotherapy with increasing trends, whereas some other cases showed fluctuation of the IRF

Table 1. Patient characteristics.

Number of patients	21
Median age (range)	41(14-62)
Gender(male/female)	16/5
Disease	
Non -Hodgkin's lymphoma	9
Hodgkin's disease	4
Peripheral T-Cell lymphoma	2
Lymphoma in leukaemic phase	1
Acute leukaemia	5
No of patients undergone harvesting procedures	12
Reason for exclusion	
Poor mobiliser	5
Medical complications	2
Incomplete data	2

levels with days post chemotherapy. However, in this study, we observed that the median day of IRF peaks wer day 14 and the median IRF value on day 14 was 0.37 (Figure 1).

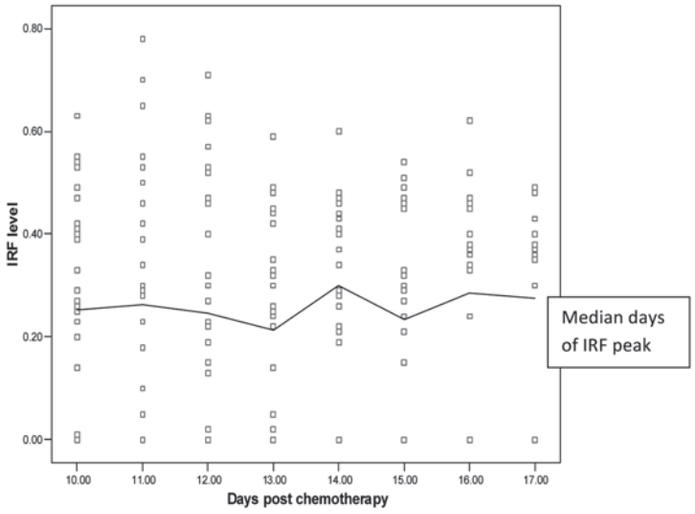

Figure 1. The IRF values of the 21 patients from day 10 to day 17 post mobilisation chemotherapy and G-CSF.

PB CD34+ cells

Peripheral blood CD34+ counts analysed from the 21 patients are shown in Figure 2. The pattern of peripheral blood CD34+ counts were shown to be in increasing trend. PB CD34+ cells rose from low to detectable levels at the time of recovery from aplasia. There was an individual variation with time as PB CD34+ cells peak. However, the trend of the plot shown in Figure 2 showed that a majority of the patients' CD34+ counts were raised from day 14 post chemotherapy and the median day of that CD34+ counts peaks was day 16. In other words, the PB CD34+ counts peaked at a median of 2 days after the IRF levels.

Correlation between IRF and PB CD34+ Cell Counts

From this study, we observed that IRF levels peaks preceded the PB CD34+ harvest by 2 days. The IRF values were more than 0.3 on the day of peripheral blood stem cell harvesting in all the 12 patients who were successfully mobilised and harvested. However, there was no significant correlation found between the IRF values and PB CD34+ cell counts.

Peripheral Blood Stem Cell Harvesting

Of the 21 patients that had undergone peripheral blood stem cell mobilisation, only 12 of them were successfully harvested. In total, 22 leukapheresis procedures were performed on these 12 patients when their PB CD34+ cells exceeded 10 CD34+ cells/μl; this was to ensure good stem cell yield. The parameters of these leukapheresis procedures are shown in Table 2. The median counts of WBCs, CD34+ cells and IRF on the day of leukapheresis were 18.2×10^9/L(range, 3.6-43.2 $\times 10^9$/L), 21.1 cell/ μl (range, 4.8-47.5 cell/ μl) and 0.47(range,0.3-0.54) respectively. The median number of CD34+ cells collected was 1.28×10^6 (range, 0.31-5.8 $\times 10^6$) per kg of patient's body weight in the apheresis product.

Even though this was a limited number of sample size, a positive correlation between PB CD34+ cells counts and harvested stem cell yields was observed which is statistically significant (r^2 0.77, p 0.021).

DISCUSSION

Predicting the timing for stem cell harvesting in autologous PBSCT remains a major problem as these patients were previously treated with chemotherapy with variable bone marrow reserve. The success of the stem cell mobilisation and the optimal timing for harvesting varies from patient to patient. In order to predict the optimal timing for stem cell harvesting, various parameters have been used such as quantification of CFU-GM (colony

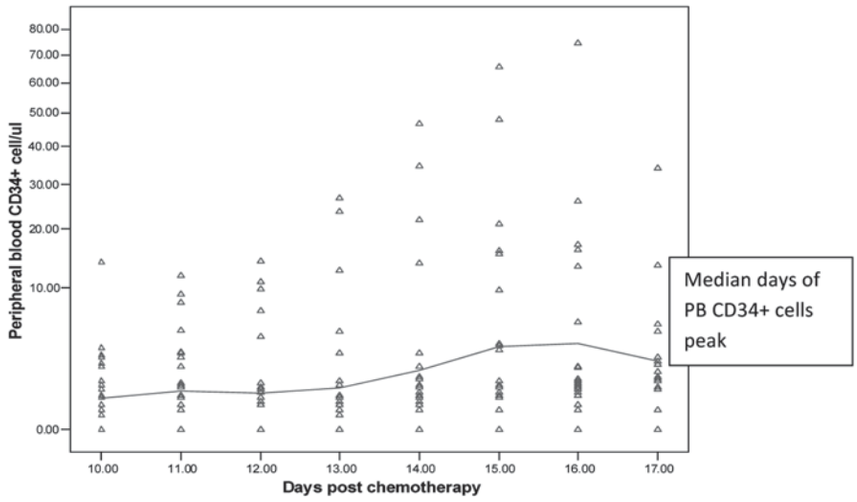

Figure 2. Peripheral blood CD34+ cells counts/µl for the 21 patients from day 10 to day 17 post chemotherapy.

Table 2. Cell counts in PB and apheresis product.

Total number of apheresis procedure	22
Number of apheresis per patient	
1	5
2	4
3	3
Blood cell counts in PB on the day of harvest*	
WBC count (x 109)/L	18.2 (3.6-43.2)
CD34 + (cell/ ul)	21.1 (4.8-47.5)
IRF	0.47 (0.3-0.54)
Stem cells harvested × 106/kg	1.28 (0.31-5.80)
Outcome of harvesting yield/ procedure	
Good stem cell yield	7
Poor stem cell yield	15

* shown is the median (range)
Good yield: harvested product > 2 × 106/ recipient BW
Poor yield: harvested product < 2 × 106/recipient BW

forming unit-granulocytes macrophages) and monitoring of white blood cells count (WBC). However, the quantification of CFU-GM requires 2 weeks to obtain the results; thus, this limits its usage as a guide for stem cell harvest [9]. Some studies have also demonstrated a poor correlation between WBC and the yield of CD34+ cells. [3,10,11,13] Nowadays, the standard parameter for guiding peripheral blood stem cell harvesting has been peripheral blood CD34+ cell counts.

Unfortunately, the practice of monitoring PB CD34+ cell counts prior to harvesting has its limitation, i.e. it is technically complex, time consuming and costly. Daily monitoring of PB CD34+ cells especially in autologous PBSCT is an expensive exercise. IRF level is instantly available as it is part of a routine full blood count. Based on our observation, IRF determination appears to be a viable approach in optimizing PBSC collection.

The presence of immature haemopoietic cells such as IRF indicates the imminent recovery of erythropoiesis after stem cell transplant therapy. However, its value may vary between patients as factors such as sex, age, diagnosis, CD34+cells, the time interval between diagnosis and transplantation may influence bone marrow recovery. Among the 21 patients recruited, whereby 205 pre-harvest tests were analysed, both the PB CD34+ cells and IRF levels varied from patient to patient. Some patients had very low IRF levels which were only detected from day 13 post chemotherapy onwards. Gowan *et al*. 1999[8], reported that there was significant variations in the time interval between the respective values found in IRF.

Remacha *et al.* (1996)[6], have shown that an increase in IRF precedes the presence of circulating PB CD34+ cells by two days in patients mobilised with chemotherapy and growth factors. In most of our cases, the IRF recovered almost at the same time as PB CD34+ cells. We also observed that the median days of IRF peaks and PBSC harvest were at day 14 and day 16 respectively, i.e. IRF peaks preceded the PB CD34+ harvest by 2 days.

Dunlop *et al.* (2006)[12], suggested that an IRF of <0.2 as a negative predictor marker of not to perform CD34 cell count as it will be below the harvestable threshold level. In contrast, the findings of the recent study revealed that stem cells were defined as harvestable, with the CD34 count being >10/µl, while the peak of IRF were all > 0.3.

Even though PB CD34+ cells measurement is an expensive, time consuming and labour-intensive method, it remains a reliable and standard tool which could predict the optimal time for collection of CD34+ cells. Therefore, PB CD34+ cells measurement would continue to be used for predicting the time to initiate the collection procedure in patients on chemotherapy-induced mobilisation. Though IRF can be performed on a routine haematology analyser without special operator skills and results are readily available, we were unable to show its correlation with the harvested stem cell yield. However, we observed that the IRF level peaked 2 days earlier than the PB CD34+ cell counts; therefore, we propose that the IRF may be used as a screening parameter to initiate the monitoring of PB CD34+ cell count, i.e. following the chemotherapy plus G-CSF mobiliszation protocol. Daily IRF may be used as a guide to monitor this group of patients. Once IRF level peaks or exceeds 0.3, then the PB CD34+ cell counts should be initiated and monitored thereafter until the day of stem cell harvesting.

In conclusion, in autologous stem cell mobilisation, we believe that IRF may be a useful screening tool to predict the rise of PB CD34+ cell counts as IRF peaks are closely related to PB CD34+ cell count and it is a simple, fast and less costly test. An IRF level of ≥0.3 may be used to initiate the quantification of PB CD34+ cell counts prior to stem cell harvest. Nonetheless, as to whether this tool is accurate and useful, this remains to be validated in a prospective study with an appropriate sample size.

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