การนับปริมาณเซลล์ที่ติด CD34 ในเลือดก่อนเก็บเซลล์ต้นกำเนิดเม็ดเลือดสามารถทำนายปริมาณเซลล์ต้นกำเนิดเม็ดเลือดที่เก็บได้

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Pre-Apheresis Peripheral Blood CD34+ Cell Counts Predict the Yield of CD34+ Cells Harvesting

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หลักการและวัตถุประสงค์: ขั้นตอนสำคัญในการปลูกถ่ายเซลล์ต้นกำเนิดเม็ดเลือดเพื่อรักษาโรคทางโลหิตวิทยาคือการเก็บเซลล์ต้นกำเนิดเม็ดเลือดให้เพียงพอโดยนิยมเก็บจากเลือด หากการนับปริมาณเซลล์ที่ติด CD34 ในเลือดก่อนเก็บเซลล์ สามารถทำนายปริมาณเซลล์ต้นกำเนิดเม็ดเลือดที่เก็บได้อย่างดี จะเป็นประโยชน์ในการเลือกวันเก็บเซลล์และปรับเปลี่ยนวิธีการกระตุ้นเก็บเซลล์ต้นกำเนิดเม็ดเลือดได้อย่างเหมาะสมดังนั้นการศึกษานี้จึงมีวัตถุประสงค์เพื่อ ศึกษาความสัมพันธ์ระหว่างปริมาณเซลล์ที่ติด CD34 ในเลือดก่อนเก็บเซลล์และปริมาณเซลล์ต้นกำเนิดเม็ดเลือดที่เก็บได้

วิธีการศึกษา: ศึกษาย้อนหลังเชิงวิเคราะห์และพรรณนาเก็บข้อมูลย้อนหลัง 7 ปี และวิเคราะห์หาความสัมพันธ์โดย Pearson's rank correlation

ผลการศึกษา: ปริมาณเซลล์ต้นกำเนิดเม็ดเลือดที่เก็บได้สัมพันธ์อย่างดีกับปริมาณเซลล์ที่ติด CD34 ในเลือดที่นับก่อนเก็บเซลล์ (r = 0.93, p < 0.001) และสามารถคำนวณโดย [ปริมาณเซลล์ต้นกำเนิดเม็ดเลือดที่เก็บได้ $(X10^6 /kg)$= 0.362 + 0.07 (ปริมาณเซลล์ที่ติด CD34 ในเลือดที่นับก่อนเก็บเซลล์) (cells/uL)]

สรุป: ปริมาณเซลล์ที่ติด CD34 ในเลือดที่นับก่อนเก็บเซลล์เป็นตัวทำนายปริมาณเซลล์ต้นกำเนิดเม็ดเลือดที่เก็บได้อย่างดีเยี่ยม สามารถใช้เป็นเครื่องมือเลือกวันเก็บเซลล์ต้นกำเนิดเม็ดเลือดได้อย่างมีประสิทธิภาพและช่วยพัฒนาการดูแลผู้ป่วย

Background and Objectives: Hematopoietic Stem Cell Transplantation (HSCT) is a standard care for many hematologic disorder patients. An essential step of HSCT is the stem cell harvesting. The Peripheral blood (PB) stem cell is the most commonly used source in adult patients. If the strong correlation between pre-apheresis PB CD34+ cells and the yield of CD34+ cells harvesting is demonstrated, this value will guide physicians to choose for an appropriate date of stem cell collection and to a modify stem cell mobilization protocol properly. This study aimed to determine the correlation between pre-apheresis peripheral blood CD34+ cell counts and the yield of CD34+ cells after harvested

Methods: This study was designed as a retrospective descriptive and analytical study. Data has been collected from medical records of all adult apheresis for 7 years. The correlation between each variable was demonstrated by Pearson's rank correlation.

Results: A total of 41 patients undergoing autologous HSCT with 71 PBSC harvesting were included. The yield of CD34+ cells harvesting were strongly correlated with PB CD34+ cells (r = 0.93, p < 0.001) and can be predicted by **[Yield of CD34+ cells harvested $(X10^6 /kg)$ = 0.362 + 0.07 (Pre-apheresis PB CD34+ cells) (cells/uL)]**

If the goal of CD34+ harvesting is at least $1 X10^6/kg$, the targetof PB CD34+ cells before stem cell collection will be at least 9 cells/uL.

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Conclusions: PB CD34+ cell counts before harvesting represents the impressive predictor of the optimal time for successful harvesting. This predictor will promote PB stem cell harvesting efficiently, and will also improve overall patient care.

Keywords: Peripheral Blood CD34+; Yield of CD34+; Predictor of Stem cell yield; Actual stem cells collected by apherresis; Stem cell collection

Introduction

The Autologous Hematopoietic Stem Cell Transplantation (AHSCT) or the Bone Marrow Transplantation (BMT) is a standard care for many hematologic disorder patients especially patients with Multiple Myeloma and Lymphoma[1-10]. The goal of HSCT is to cure the disease or to prolong disease remission and to improve overall survival rate[1-10]. An important step of HSCT is stem cell harvested (stem cell collection) from the patients themselves (Autologous HSCT) or HLA matched donor (Allogeneic HSCT). The poor mobilization is a common reason for not performing HSCT[11]. The Peripheral blood (PB) stem cell is the most commonly used source in adult patients. Routinely stem cell collection based on only the number of days after G-CSF administration without good predictor is causing inadequate collected CD34+ stem cell because of too early or too delay collection, and perform collection in poor mobilized patients. Each apheresis consumes multiple resources. A good prediction for the yield of CD34+ harvesting will promote the cost-effectiveness resource utilization and will maximize benefits.

Pre-apheresis PB CD34+ cells counts before harvesting is a rapid inexpensive tool. If the correlation between it and the yield of CD34+ cells harvesting is high, this tool will guide the starting and the ending date of stem cell collection. If pre-apheresis PB CD34+ cells are low, the mobilization protocol will be modified by increasing doses of granulocyte-stimulating factor (G-CSF) or by adding plerixafor in combination.

This study aimed to determine the correlation between pre-apheresis PB CD34+ cell counts and the yield of CD34+ cells after harvested

Materials and Methods

Study Design

A retrospective descriptive and analytical study of all adult transplanted patients in Srinagarind hospital, Thailand has been observed. Data has been collected from medical records for 7 years (January 1st 2010 to December 31st 2016). Every stem cell harvesting was included and explored.

Methods

Stem cell collections were performed by using COBE spectra (Cobe laboratories) or Spectra Optia (Terumo BCT) continuous flow cell separator. Peripheral blood was taken immediately before apheresis, was stored at room temperature and was tested within 24 hours after collection. CD34+ cell concentration in peripheral blood was determined by Stem-Kit[TM] reagent (Beckman Coulter, Miami FL). Briefly, EDTA blood was labelled with fluorescent antibodies against CD34 and CD45 and stained with 7-AAD to determine cell viability and red cells (RBCs) were lysed with an ammonium chloride buffer. Single-platform, microbead-based cell counting procedures were also accomplished using flow count beads. The measurements were performed on the flow cytometer (Beckman Coulter FC500)[12].

Statistical Analysis

Patients' data was analysed by statistical software STATA version 10.0. Patients' information including categorical and continuous data was summarized separately and differently. For categorical data, the information was presented in a form of percentage, while two sets of statistical tools, the median performed with range and the mean performed with standard deviation (SD) were applied to interpret continuous data. A magnitude of difference in prevalence was presented as percentage, a p less than 0.05 is considered statistically significant. The Correlation between each variable was demonstrated by Pearson's rank correlation analysis with correlation coefficient (r) and p.

Ethical consideration

This study protocol including with the study information and case record form was approved and accepted by the Ethic committee for Research in Human Subjected at Srinagarind hospital.

Results

A total of 41 patients undergoing autologous HSCT with 71 PBSC harvesting were included. Clinical profiles were as follows: male patients represented 80% with a median age of 49 years (range 18-65 years) having multiple myeloma (MM) (80%), while 20% were relapsed or refractory lymphoma. Patients were mainly treated by intensive standard chemotherapy until achieve at least very good partial response (VGPR) for MM and complete remission (CR) for lymphoma before stem cell collection. All cases were mobilized stem cell by using G-CSF alone (10ug/kg/day). Stem cell harvesting was started on the 4th or 5th day after G-CSF. Median yield of stem cell harvesting per patients was 6.15 $X10^6$ /kg (range 1.17-23.53 $X10^6$ /kg). Pre-apheresis complete blood count (CBC) and PB CD34+ cells counts were routinely measured and recorded. PBSC were preserved in standard dimethyl sulfoxide (DMSO) solution before stem cell infusion.

PB CD34+ cells were strongly correlated with the yield of CD34+ cells after harvested (r = 0.93, p < 0.001) as demonstrated in **Figure 1**.
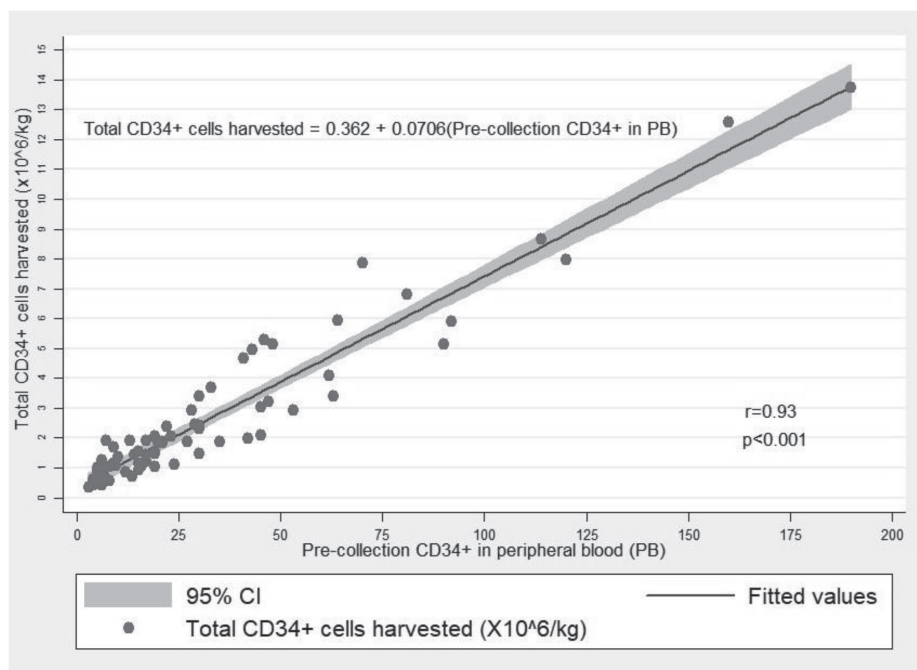

Figure 1 correlation between pre-apheresis peripheral blood CD34+ cell counts and the yield of harvested CD34+ cells

The yield of stem cell harvested from each apheresis was predicted by this following equation;

$$[\text{Yield of CD34+ cells harvested} = 0.362 + 0.07 \text{ (Pre-apheresis PB CD34+ cells)}]$$

$(\text{X}10^6 \text{/kg})$ \hspace{6cm} (cells/uL)

If the goal of CD34+ harvesting per each apheresis is at least $1\text{X}10^6$/kg, the calculated PB CD34+ cells before stem cell collection will be at least 9-10 cells/uL. In the same way, if the target of CD34+ harvesting is $2\text{X}10^6$/kg, the target PB CD34+ will be higher than 20 cells/uL.

The correlation was obviously seen on the day before harvesting and 1st day of harvesting (r = 0.91 and 0.96). Moreover, PB CD34+ cells counts on the 1st day of harvesting were moderately correlated with total PBSC harvested (r = 0.78, p < 0.001).

Contrarily, WBC counts before a stem cell collection did not correlate with the yield of CD34+ cells harvesting (r = 0.10, p = 0.002) as demonstrated in Figure 2.
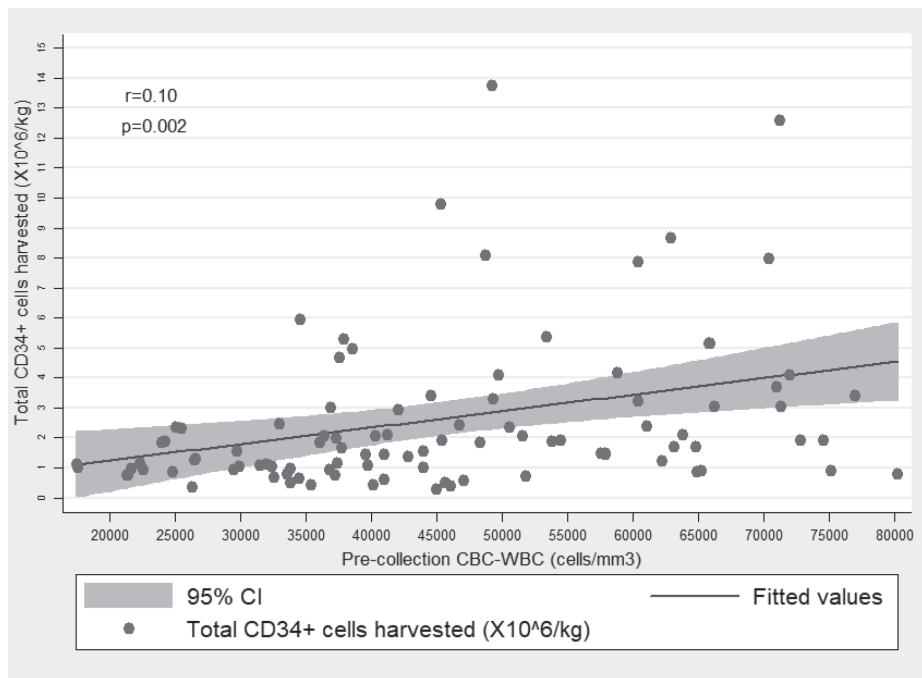

Figure 2 correlation between pre-collection CBC-WBC and the yield of harvested CD34+ cells

Discussion

It is clear that the number of CD34+ cells in PB was closely correlated with the yield of CD34+ cells harvesting. Similar results have been reported by many studies[13-28] but the method of CD34+ cells count between each laboratories were different. Therefore, the correlation equation may have changed depending on counting methods.

This study noticeably provided the correlation equation to calculate the yield of CD34+ cells harvest-ing by pre-apheresis PB CD34+ cells. The minimal threshold of CD34+ for successful engraftment of HSCT is $2\text{X}10^6$/kg[11,13,14,29]. If the goal of CD34+ cells harvesting is at least $1\text{X}10^6$/kg per apheresis, the minimal target of pre-collecting PB CD34+ cells will be 8-9 cells/uL(calculated by our equation). Minimal requirement of PB CD34+ cells before harvesting in previous study is varies between 8-15 cells/uL[11, 13, 21].

This result will be highly benefited when CD34 enumeration can be performed within 2 hours after

sample collection[12]. Continuous developing of CD34 enumeration should be performed.

The major limitations of this study were the retrospective nature of the study and the small number of participants due to the fact that Srinagarind HSCT unit has just been established.

The strengths in this study are the homogeneity of stem cell collection, mobilization and CD34+ cell measurement, and additionally the discovery of a simplified applicable formula for calculated the yield of stem cells harvesting.

Conclusions

Monitoring of CD34+ cells in peripheral blood before stem cell collection is an impressive predictor of the optimal time for adequate harvesting.This prediction method will promote PB stem cell harvesting efficiently, minimize harvesting-related adverse events, decrease unnecessary prolongation of apheresis and improve patient care.

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