

Clinical Usefulness of the Hematopoietic Progenitor Cell Counts in Predicting the Optimal Timing of Peripheral Blood Stem Cell Harvest

Although enumeration of CD34+ cells in the peripheral blood (PB) on the day of apheresis predicts the quantity of those cells collected, the flow cytometric techniques used are complex and expensive, and several hours are required to obtain the result in the clinical practice setting. The Sysmex SE-9000 automated haematology analyzer provides an estimate of immature cells, called hematopoietic progenitor cells (HPC). The aim of this study was to evaluate the clinical usefulness of HPC in predicting the optimal timing of peripheral blood progenitor cells (PBPC) harvest. Studies were performed on 628 aphereses from 160 patients with hematologic or solid malignancies. Spearman's rank statistics was used to assess correlation between HPC, WBC, mononuclear cells (MNC), and CD34+ cells. A receiver operating characteristic (ROC) curve was drawn for cutoff value of HPC, and predictive values of the chosen cutoff value of HPC for different target CD34+ cell collections were calculated. The PB HPC had a stronger correlation ($\rho=0.592$, $p<0.001$) with collected CD34+ cells than did PB WBC and PB MNC. The ROC curve showed that the best cutoff value of HPC was $50 \times 10^6/L$ for the target CD34+ cells $\geq 1 \times 10^6/kg$ with sensitivity of 75%. Positive and negative predictive values of HPC $\geq 50 \times 10^6/L$ for CD34+ cells $\geq 1 \times 10^6/kg$ were 59.7% and 81.1%, respectively. In the clinical practice setting, applying variable cutoff values of HPC would be a useful tool to predict the optimal timing of PBPC collection.

Key Words: Hematopoietic Stem Cells; Leukapheresis; Antigens, CD34; Transplantation, Autologous

Jae-Lyun Lee, Sung-Bae Kim,
Gyeong-Won Lee, Min-Hee Ryu,
Eun-Kyeong Kim, Shin Kim, Woo-Kun Kim,
Jung-Shin Lee, Keon Uk Park*,
Cheolwon Suh

Department of Medicine, University of Ulsan
College of Medicine, Asan Medical Center, Seoul;
*Department of Internal Medicine, Dongguk
University College of Medicine, Gyeongju, Korea

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Address for correspondence

Cheolwon Suh, M.D.
Department of Medicine, University of Ulsan
College of Medicine, Asan Medical Center, 388-1
Pongnap-dong, Songpa-gu, Seoul 138-040,
Korea
Tel : +82-2-3010-3209, Fax : +82-2-3010-6961
E-mail : csuh@amc.seoul.kr

INTRODUCTION

High-dose chemotherapy with autologous stem cell transplantation is increasingly used in a wide range of hematologic and solid malignancies and several trials have suggested that this strategy is effective in patients with aggressive lymphoma (1) and multiple myeloma (2). Peripheral blood progenitor cells (PBPC) have almost completely replaced bone marrow as the source of stem cells because of easier accessibility, faster engraftment, and possibly lower tumor contamination (3-5). As a result of this change in practice, there are currently an increasing number of patients undergoing PBPC mobilization therapy and subsequent leukapheresis.

Successful autologous peripheral blood stem cell transplantation (PBSCT) depends on the infusion of an adequate number of PBPC to achieve rapid and durable hematologic recovery. The infusion of $\geq 2.5 \times 10^6$ CD34+ cells per kg of body weight (/kg) is generally considered to be sufficient to induce stable hematologic engraftment within a reasonable time. The transplantation of $\geq 5 \times 10^6$ CD34+ cells/kg results in a faster hematopoietic recovery, particularly of the megakaryocytic lineage (6).

The timing of PBPC collection is important to maximize the number of progenitors harvested with the fewest apheresis procedures. For operational and economical efficiency, it would clearly be beneficial if sufficient PBPC could be obtained from the fewest collections and the optimal timing of these leukaphereses could be reliably predicted. A further benefit of a higher CD34+ cell yield per single apheresis is reduction in the total volume of the progenitor cell component. Besides the decrease in the contaminating RBCs within the graft (7), a reduction in the total volume of the PBPC component may reduce the amount of dimethyl sulfoxide required for cryopreservation (8). Both factors diminish the risk of adverse side effects related to the graft transfusion.

The most reliable time for harvesting PBPC is yet to be determined. Several different predictors for PBPC yield have been used for the timing of apheresis. Circulating progenitor cells (CFU-GM) cannot be used for this purpose because it takes about 2 weeks to form enumerable colonies and is difficult to standardize. Following a chemotherapy nadir, the peripheral blood (PB) WBC count has been used to indicate when leukapheresis should be commenced. The collections are usually initiated when the WBC count recovers to greater than

$1 \times 10^9/L$ (9). However, other studies have suggested that waiting until the WBC count is greater than $2-10 \times 10^9/L$ may be advantageous (10, 11). Furthermore, there is little correlation between the PB WBC count and the number of CD34+ cells in a leukapheresis product, resulting in inefficient collections and increasing the overall cost of obtaining PBPC for the transplant (12-15). While enumeration of CD34+ cells in the PB before apheresis predicts the quantity of those cells collected (16-18), the flow cytometric techniques used are complex and expensive. Also, several hours are usually required to obtain the result, which complicates patient management in the clinical practice setting. A simple, faster, and less expensive alternative method should therefore be pursued, especially in the countries with limited medical budget.

Sysmex has developed an automated hematology analyzer (Sysmex SE9000, TOA Medical Electronica Co. Ltd. Japan) that detects a small population of immature white blood cells, hematopoietic progenitor cells (HPC), with the use of special equipment called the immature myeloid information (IMI) channel (19). We have previously reported on the preliminary data, which included 32 patients, of the correlation between the number of PB HPC and collected CD34+ cells (20). In the present study, we evaluated potential usefulness of PB HPC count in the clinical practice through the analysis of the data on 160 consecutive patients. We report the kinetics of PB HPC and CD34+ cells in apheresis products, the correlation between the PB HPC and collected CD34+ cells, sensitivity, specificity, and predictive values of HPC for variable target of CD34+ cells collection, and the clinical usefulness of HPC to judge apheresis commencement.

MATERIALS AND METHODS

Patients

Between July 1998 and April 2002, 160 consecutive patients with hematologic or solid malignancies who were eligible for autologous PBST underwent PBPC harvests at our institution. Prior to beginning PBPC mobilization and harvest, all patients had given signed informed consent approved by the institutional review board of Asan Medical Center.

PBPC mobilization and harvest

Forty-one patients with multiple myeloma, 31 patients with lymphoma, 20 patients with breast cancer, and 3 patients with other malignancies were mobilized with cyclophosphamide (Cy) $2-4 g/m^2$ with G-CSF (Neutrogin™, Choongwae Ltd, Seoul, Korea). Seven patients were primed with G-CSF alone. Others were mobilized with various chemotherapy regimens that were chosen both for their efficacy against the patients' disease and their ability to induce a WBC rebound following marrow aplasia. For patients mobilized with Cy or chemo-

therapy, the first dose of G-CSF was given subcutaneously at a dose of $5-10 \mu g/kg/day$ from the day of nadir of the WBC count after Cy or other chemotherapy had ended and continued until the day before the last leukapheresis. The first PBPC harvest was performed on the day when the WBC count exceeded $10.0 \times 10^9/L$ or MNC count exceeded $1.0 \times 10^9/L$ for patients mobilized with Cy or chemotherapy. It was undertaken on the 4th to 5th day of G-CSF administration if no chemotherapy was used.

PBPC were collected with a continuous-flow blood cell separator (Fenwal CS3000 plus, Baxter healthcare, Deerfield, IL, U.S.A.). Each apheresis procedure was performed for approximately 2 to 4 hr, processing 10-14 L of whole blood volume. The total MNC count and CD34+ count of the leukapheresis product were monitored daily following each collection. Leukapheresis was continued until analysis of the component confirmed the collection of $\geq 5.0 \times 10^6$ CD34+ cells/kg.

Measurement of HPC

Enumeration of HPC was performed using the Sysmex SE 9000. HPC were detected in the IMI channel. Detection of HPC was made possible by the combination of a special reagent system and direct current (DC)/radiofrequency (RF) biosensors. The lysis reagent (Stromatolyser-IM) contains detergents that are capable of lysing more mature WBC because of their higher membrane lipid content while HPC remains relatively intact. Because various types of immature WBC react differently to the reagent, they also occupy distinct areas on the bivariate matrix of the IMI scattergram (19). Using the purified CD34+ cells, the lower DC and RF signal were previously identified for the HPC detection area. HPC were reported both as an absolute number and a percentage of the WBC in the sample.

Measurement of CD34+ cells

The quantities of CD34+ cells in the leukapheresis component were determined by flow cytometry using FACScan (Becton Dickinson, Palo Alto, CA, U.S.A.). After lysis of RBCs in an ammonium chloride lysis solution and washing with phosphate-buffered saline with 0.5% human serum albumin, 100 μL of cell suspension was stained with phycoerythrin (PE)-conjugated monoclonal anti-CD34 antibody (HPCA-2, Becton Dickinson) and fluorescein isothiocyanate (FITC)-conjugated anti-CD14 antibody (Leu-M3, Becton Dickinson) at the concentrations recommended by the manufacturer. After incubation for 25 min at $4^\circ C$ in the dark, additional cells were stained with Leu-M3 and PE-conjugated IgG1 (Becton Dickinson) as a negative isotope control. Then the cells were washed twice and resuspended and examined with a FACScan. A total of 1.0×10^5 cells were analyzed by the FACScan research software (Becton Dickinson).

Measurement of nucleated cells in the PB and apheresis products

WBC count in the sample was determined with Sysmex SE9000. Differential counts were done microscopically on a Wright stained smear. The mononuclear cell count was obtained by multiplying the number of leukocytes with the sum of the percentage of lymphocytes and monocytes from the differential count.

Statistics

Patients' characteristics and apheresis components are described using summary statistics as median values and ranges. Correlations between the logarithm of the number of CD34+ cells and HPC cells, WBC, MNC in the PB and apheresis products were assessed using linear regression and Spearman's rank correlation coefficient (ρ). To assess the feasibility of HPC as a diagnostic test for predicting the ability to achieve target CD34+ cells of $1 \times 10^6/\text{kg}$ and 2×10^6 cells/kg, the receiver operating characteristic (ROC) curve was used. We calculated positive and negative predictive values of HPC, utilizing several different cutoff points for HPC. Statistical analysis was performed with SPSS for Windows V.10.0 (SPSS Inc, Chicago, IL, U.S.A.). Significance levels were set at 0.05.

RESULTS

Patient characteristics

Patients' characteristics are given in Table 1. There were 80 women and 80 men. The median age was 45 yr ranging from 15 to 68 yr. The patients with non-Hodgkin's lymphoma comprised 40 percent of the patients (64/160). Other malignancies included multiple myeloma in 51 patients, breast cancer in 33, acute myelogenous leukemia in 5, medulloblastoma in 4, ovarian cancer in 2, and acute lymphocytic leukemia in 1 patient. Forty-one (29.3%) of 160 patients had been given 7 or more cycles of chemotherapy, and 104 (68%) patients had a history of prior exposure to alkylating agents, which included melphalan, BCNU, and cyclophosphamide. Twenty-two (14%) of 160 patients had previously undergone axial skeletal radiotherapy.

Table 1. Patient characteristics

| | |
|--|------------|
| Age (yr), median (range) | 45 (15-68) |
| Male:female | 80:80 |
| Disease | |
| Breast cancer | 33 |
| NHL | 64 |
| MM | 51 |
| Others (AML, medulloblastoma, ovary cancer, ALL) | 12 |
| Previous axial skeletal RT, No (%) | 22 (14%) |
| Cycles of prior chemotherapy, median (range) | 5 (2-30) |
| Number of prior chemotherapy regimen | |
| 1 | 83 (53%) |
| 2 | 56 (35%) |
| ≥ 3 | 19 (11%) |
| Previous exposure to alkylating agents No. (%) | 104 (68%) |

NHL, non-Hodgkin's lymphoma; MM, multiple myeloma; AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; RT, radiotherapy.

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Mobilization and collection of PBPC

A total of 628 leukaphereses were done in 160 patients (Table 2). The median number of CD34+ cells and MNC in a leukapheresis product was $0.65 \times 10^6/\text{kg}$ (range, 0.01-25.62) and $1.40 \times 10^8/\text{kg}$ (range, 0.14-6.74), respectively. The percentages of leukapheresis products reaching the target yield

Table 2. Mobilization methods, peripheral blood nucleated cell counts, and results of CD34+ cell collection

| | |
|--|---|
| Mobilization therapy | |
| Cyclophosphamide+G-CSF | 92 |
| Chemotherapy+G-CSF | 61 |
| G-CSF alone | 7 |
| Total number of leukapheresis procedures | 628 |
| Number of leukapheresis procedure per person | 4 (2-9)* |
| Number of HPC in PB | $46.64 \times 10^6/\text{L}$ (0-3052.21) |
| Number of WBC in PB | $13.10 \times 10^9/\text{L}$ (0.80-55.50) |
| Number of MNC in PB | $1.67 \times 10^9/\text{L}$ (0.10-10.96) |
| Number of CD34+ cell in a leukapheresis | $0.65 \times 10^6/\text{kg}$ (0.01-25.62) |
| Number of MNC in a leukapheresis | $1.40 \times 10^8/\text{kg}$ (0.14-6.74) |
| Number of total CD34+ cell collected | $5.43 \times 10^6/\text{kg}$ (0.03-60.16) |
| Number of total MNC cell collected | $6.42 \times 10^8/\text{kg}$ (0.72-17.73) |

*Median (range). G-CSF, granulocyte-colony stimulating factor; HPC, hematopoietic progenitor cell; MNC, mononuclear cell.

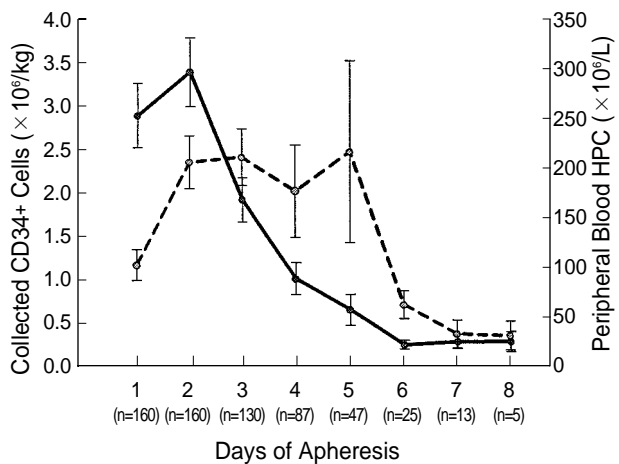


Fig. 1. The kinetics of CD34+ cells in an apheresis product and the peripheral blood HPC. Solid line, CD34+ cells; dashed line, HPC (mean \pm SE).

of CD34+ cells $\geq 1 \times 10^6/\text{kg}$ and $2 \times 10^6/\text{kg}$ were 42.2% and 26.6%, respectively. The kinetics of HPC in the PB and CD34+ cells in a leukapheresis product over the course of collection for all the patients are depicted in Fig. 1. The kinetics of HPC in PB did not seem to parallel that of CD34+ cell in apheresis products. A median of 5.43×10^6 CD34+ cells/kg (range, 0.03-60.16) was harvested by a median of 4 (range, 2-9) apheresis procedures. CD34+ cells greater than $2.5 \times 10^6/\text{kg}$ were harvested from 113 (71%) patients. In 47 patients (29%) who failed to attain 2.5×10^6 CD34+ cells/kg with a median of 4 apheresis procedures (range, 2-8), there were no

medical complications that led to discontinuation of apheresis, and harvests were discontinued when they were not likely to yield $\geq 0.2 \times 10^6/\text{kg}$ CD34+ cells per day. Thirty-four (72.3%) of 47 patients who failed to yield $\geq 2.5 \times 10^6$ CD34+ cells/kg underwent ≥ 4 apheresis procedures.

The correlation between cell counts in the PB and the yield of CD34+ cells

The median number of PB HPC on the day of leukapheresis was $46.6 \times 10^6/\text{L}$ (range, 0-3052.2, Table 2). The corre-

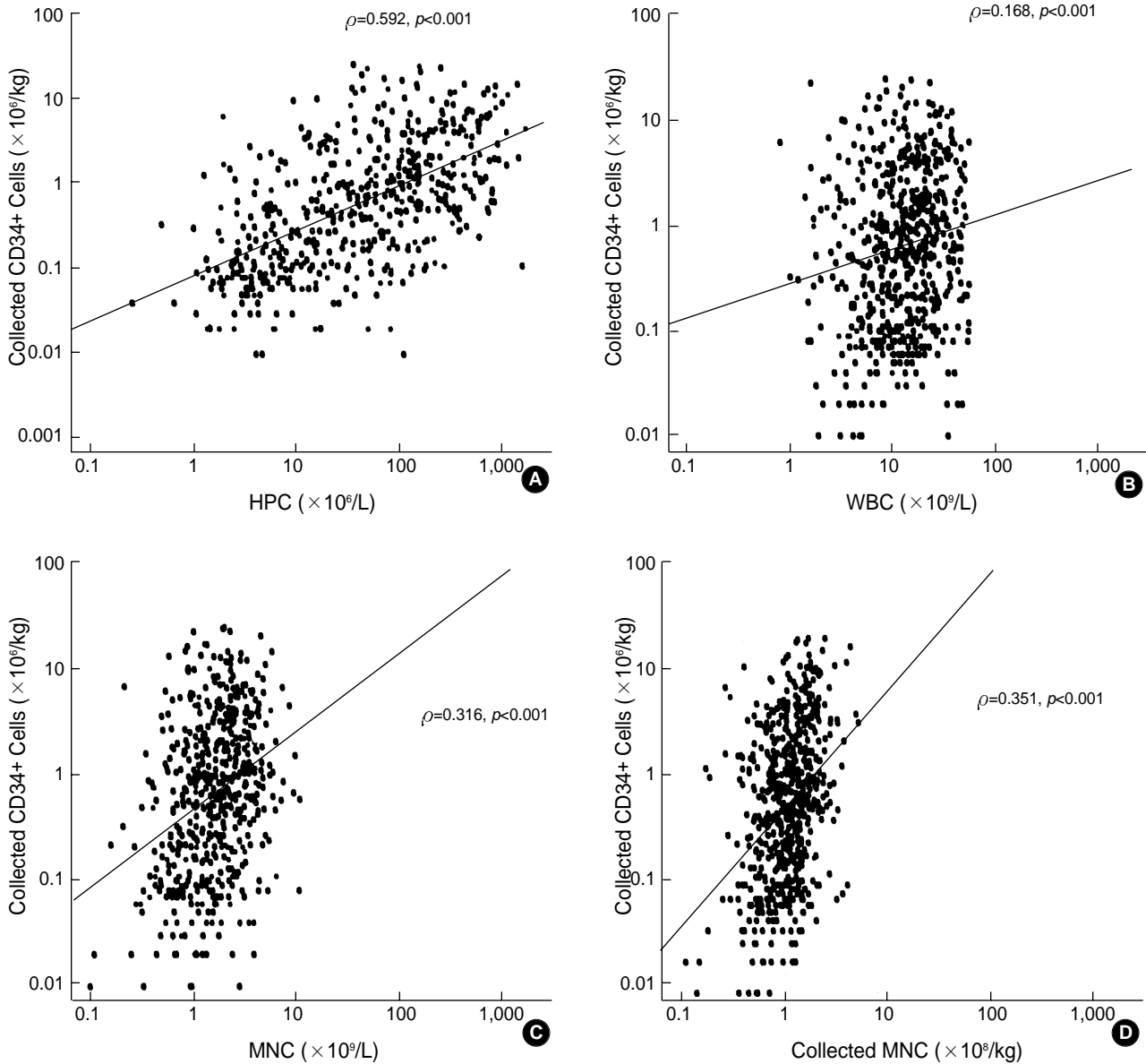


Fig. 2. The relationship between the collected CD34+ cells and peripheral blood nucleated cell counts. Plotted are the yield of CD34+ cells ($\times 10^6/\text{kg}$) in an apheresis product (Y-axis) versus: (A) the number of peripheral blood HPC cells ($\times 10^6/\text{L}$); (B) the number of peripheral blood WBC counts ($\times 10^9/\text{L}$); (C) the number of peripheral blood MNC counts ($\times 10^9/\text{L}$); and (D) the number of collected MNC ($\times 10^9/\text{kg}$) in an apheresis product (\times -axis) in all apheresis procedures.

sponding median numbers of peripheral blood WBC and MNC on the day of leukapheresis were $13.10 \times 10^9/L$ (range, 0.80-55.50) and $1.67 \times 10^9/L$ (range, 0.1-10.96), respectively.

The relationship between PB nucleated cell counts on the day of apheresis and the number of CD34+ cells in an apheresis product is depicted in Fig. 2. The number of HPC in the PB had a stronger correlation ($\rho=0.592, p<0.001$) with collected CD34+ cell counts than did PB WBC ($\rho=0.168, p<0.001$) and PB MNC ($\rho=0.316, p<0.001$) counts. There was a weak correlation between collected MNC counts with collected CD34+ cell counts ($\rho=0.351, p<0.001$).

Sensitivity, specificity, and predictive values of PB HPC counts

We calculated the HPC level in PB as a diagnostic tool for predicting a target yield of CD34+ cells of at least $1 \times 10^6/kg$ and $2 \times 10^6/kg$. To set the cutoff value, we used a ROC curve. The ROC curve graphically portrays the trade-offs involved between either test's sensitivity and specificity. We plotted sensitivity as a function of 1-minus-specificity, false positive rate. The ROC curve revealed that the best cutoff point for the most adequate sensitivity and specificity was $50 \times 10^6/L$ for target CD34+ cells $\geq 1 \times 10^6/kg$ and $55 \times 10^6/L$ for target CD34+ cells $\geq 2 \times 10^6/kg$ (Fig. 3). The sensitivity and

specificity of that cutoff value are listed in Table 3. The cutoff value of PB HPC and the ROC curves for each target PBPC yield is very close, so the ability of PB HPC to discriminate between PBPC yield $\geq 1 \times 10^6$ CD34+ cells/kg and $\geq 2 \times 10^6$ CD34+ cells/kg is negligible.

We calculated a positive and a negative predictive value of HPC using several different cutoff values (80, 70, 60, 50, 20, 10, and $5 \times 10^6/L$). These predictive values are shown in Table 4. For patients with HPC counts $\geq 50 \times 10^6/L$, 59.7% of 263 collections reached the target CD34+ cells $\geq 1 \times 10^6/kg$. The

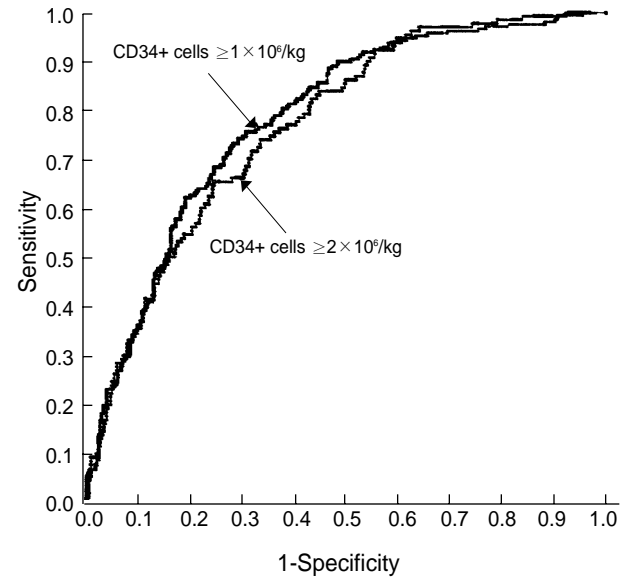


Fig. 3. ROC curves for the peripheral blood HPC to obtain $\geq 1 \times 10^6$ CD34+ cells/kg (AUC: 0.783, $p<0.001$) and $\geq 2 \times 10^6$ CD34+ cells/kg (AUC: 0.768, $p<0.001$). The curves have a similar shape, so the ability to discriminate between PBPC yield $\geq 1 \times 10^6$ CD34+ cells/kg and $\geq 2 \times 10^6$ CD34+ cells/kg is negligible.

Table 3. The recommended cutoff value of HPC for different target collection of CD34+ cells with the best sensitivity and specificity drawn from the ROC curve

| Target CD34+cells | Cutoff value of HPC | Sensitivity | Specificity |
|-------------------------|---------------------|---------------------|--------------------|
| $\geq 1 \times 10^6/kg$ | 50 | 75.5% (69.9-80.6%)* | 68.4% (63.2-73.1%) |
| $\geq 2 \times 10^6/kg$ | 55 | 75.4% (66.9-82.3%) | 62.3% (57.8-67.5%) |

*95% confidence interval.

Table 4. The positive and negative predictive values (PV) of HPC for different target collection of CD34+ cells

| Cutoff value of HPC (μL) | | Target CD34+ cells | |
|---------------------------------|-------------|-------------------------------|------------------------------|
| | | $\geq 1 \times 10^6/kg$ | $\geq 2 \times 10^6/kg$ |
| 80 | Positive PV | 139/217 (64.1%) (57.7-70.5%)* | 91/217 (41.9%) (35.3-48.5%) |
| | Negative PV | 247/316 (78.2%) (73.6-82.7%) | 277/316 (87.7%) (84.1-91.3%) |
| 70 | Positive PV | 143/227 (63.0%) (56.7-69.3%) | 94/227 (41.4%) (35.0-47.8%) |
| | Negative PV | 241/306 (78.8%) (74.2-83.4%) | 270/306 (88.2%) (84.6-91.8%) |
| 60 | Positive PV | 150/241 (62.2%) (55.8-68.3%) | 97/241 (40.2%) (34.1-46.8%) |
| | Negative PV | 219/270 (81.1%) (76.4-85.8%) | 240/270 (88.9%) (85.2-92.6%) |
| 50 | Positive PV | 157/263 (59.7%) (53.8-65.6%) | 100/263 (38.0%) (32.1-43.9%) |
| | Negative PV | 219/270 (81.1%) (76.4-85.8%) | 240/270 (88.9%) (85.2-92.6%) |
| 20 | Positive PV | 184/336 (54.8%) (49.5-60.1%) | 117/336 (34.8%) (29.7-39.9%) |
| | Negative PV | 173/197 (87.8%) (83.2-92.4%) | 184/197 (93.4%) (89.9-96.9%) |
| 10 | Positive PV | 195/390 (50.0%) (45.0-55.0%) | 126/390 (32.3%) (27.7-36.9%) |
| | Negative PV | 130/143 (90.9%) (86.2-95.6%) | 139/143 (97.2%) (94.5-99.9%) |
| 5 | Positive PV | 200/442 (45.2%) (40.6-49.8%) | 127/442 (28.7%) (24.5-32.9%) |
| | Negative PV | 83/91 (91.2%) (85.4-97.0%) | 88/91 (96.7%) (93.0-100.0%) |

*95% confidence interval.

Table 5. Clinical implications for peripheral blood progenitor cells collection where the decision to commence apheresis is modified by peripheral blood WBC, MNC, or HPC

| Parameters of decision | | CD34+ cells $\geq 1 \times 10^6/\text{kg}$ | CD34+ cells $< 1 \times 10^6/\text{kg}$ |
|------------------------------------|-------------|--|---|
| WBC $\geq 5 \times 10^9/\text{L}$ | Yes (n=457) | 204 (44.6%)*(88.7%)* | 253 (55.4%) (84.3%) |
| | No (n=73) | 26 (35.6%) (11.3%) | 47 (64.4%) (15.7%) |
| WBC $\geq 10 \times 10^9/\text{L}$ | Yes (n=332) | 161 (48.5%) (70%) | 171 (51.5%) (57.0%) |
| | No (n=198) | 69 (34.8%) (30%) | 129 (65.2%) (43.0%) |
| MNC $\geq 1 \times 10^9/\text{L}$ | Yes (n=396) | 196 (49.5%) (85.2%) | 200 (50.5%) (66.7%) |
| | No (n=134) | 34 (25.4%) (14.8%) | 100 (74.6%) (33.3%) |
| HPC $\geq 10 \times 10^6/\text{L}$ | Yes (n=339) | 178 (52.5%) (94.2%) | 161 (47.5%) (59.4%) |
| | No (n=121) | 11 (9.1%) (5.8%) | 110 (90.9%) (40.6%) |
| HPC $\geq 20 \times 10^6/\text{L}$ | Yes (n=296) | 166 (56.1%) (87.8%) | 130 (43.9%) (48.0%) |
| | No (n=164) | 23 (14.0%) (12.2%) | 141 (86.0%) (52.0%) |
| HPC $\geq 50 \times 10^6/\text{L}$ | Yes (n=231) | 143 (61.9%) (75.7%) | 88 (38.1%) (32.5%) |
| | No (n=229) | 46 (20.1%) (24.3%) | 183 (79.9%) (67.5%) |

*Percent within the row; †Percent within the column.

positive predictive values of different cutoff points of PB HPC ranging from 5 to $80 \times 10^6/\text{L}$ increased from 45.2% to 64.1%; 16-fold increase in HPC elevated positive predictive value by just 18.9%. For patients with HPC values $< 50 \times 10^6/\text{L}$, 81.1% of 270 patients did not yield target CD34+ cell $\geq 1 \times 10^6/\text{kg}$. When we set $2 \times 10^6/\text{kg}$ CD34+ cells/kg as the target yield, the negative predictive value increased up to 89%, but the positive predictive values decreased below 40%.

Use of PB nucleated cells to decide when to commence apheresis

Excluding the patients who were mobilized with G-CSF alone and those whose PB nucleated cell counts were not fully available, 6 options for deciding when to start PBPC collection were considered (Table 5). The first option was to make a decision to start apheresis on the basis of WBC count $\geq 5 \times 10^9/\text{L}$. Fifty-five percent (253/457) of the patients with WBC count $\geq 5 \times 10^9/\text{L}$ had poor CD34+ cell yields. Positive predictive value was just about 45%. False negative rate was 35.6%, and only 15.7% of harvests that proved to be inadequate would be avoided. The second option was to start apheresis when WBC counts were above $\geq 10 \times 10^9/\text{L}$. At this threshold, 48.5% of harvests yielded CD34+ cells $\geq 1 \times 10^6/\text{kg}$, and 43% of harvests with poor yield would be avoided. However, 30% of yields greater than $1 \times 10^6/\text{kg}$ CD34+ cells would be missed. The third option was the use of PB MNC counts. When the decision to apherese was taken at the threshold of $1 \times 10^9/\text{L}$, 50.5% of the patients who fitted the MNC criteria had poor PBPC yields, and 33.3% of harvests that proved to be inadequate would be avoided. Positive predictive value was 49.5% and false negative rate was 25.4%. The fourth option was to base the decision to commence apheresis on the PB HPC count. At a cutoff value of $10 \times 10^6/\text{L}$, positive predictive value was 52.5% with false negative rate of 9.1%, which was the lowest among other options.

At a cutoff value of $20 \times 10^6/\text{L}$, 43.9% of the patients who met the criteria had poor PBPC yields, and 52.0% of aphereses that proved to be inadequate would be avoided. The last option was to commence apheresis on the day of PB HPC count $\geq 50 \times 10^6/\text{L}$. Although it had the highest positive predictive value, 24.3% of yields greater than $1 \times 10^6/\text{kg}$ CD34+ cells would be missed if aphereses were not performed because of PB HPC level $< 50 \times 10^6/\text{L}$. This option had the lowest false positive rate, and 67.5% of harvests with poor yield would be avoided.

DISCUSSION

The decision when to start leukapheresis is a critical issue for the efficient and cost-effective collection of adequate PBPC for transplantation. Many transplantation centers have used the PB CD34+ cell counts to initiate harvesting and predict yields (12, 15, 21, 22). This could be the most precise predictor of CD34+ cell yields, but the correlation coefficients between PB CD34+ cells and CD34+ cells in a leukapheresis product were variable, ranging from 0.57 to 0.95 (12, 23-25). These relatively inconsistent correlations can be caused by technical difficulties in the harvesting procedure or inaccuracies in the blood CD34+ measurements, especially when the PB CD34+ percentage is low. In addition, the techniques to enumerate CD34+ cells are complex, expensive, require technical skills, and reporting the results may take several hours, often resulting in a 1-day delay before apheresis can be started in the clinical practice setting.

We evaluated the clinical usefulness of the HPC count, enumerated by Sysmex SE-9000 automated hematology analyzer, for optimizing stem cell yields. This is rapid and incurs no cost beyond that of a blood count. The detection of HPC is possible through the IMI channel that uses DC and RF to count cells after incubation with a specific lysate designed to lyse red cells and mature white blood cells, leaving only imma-

ture forms to be counted (19, 26, 27). Previous studies reported a strong correlation between PB HPC and PB CD34+ cell counts (25, 28), and we have reported a strong correlation between HPC enumerated by Sysmex SE-9000 and CD34+ cells in the leukapheresis products from data on 32 patients (20). This strong correlation led us to investigate the clinical usefulness of HPC as a screening test to decide when to commence PBPC collection.

In the present study, we confirmed that there are statistically significant but weak correlations between PB WBC or MNC and collected CD34+ cell count. The correlation between PB HPC and CD34+ cells in an apheresis product was higher than that between WBC or MNC and CD34+ cells. However, the kinetics of PB HPC and CD34+ cells collected did not have a similar configuration. To translate these findings into the clinical practice setting, we used a ROC curve to determine the best cutoff value of PB HPC for predicting a target yield of CD34+ cells (≥ 1 or $2 \times 10^6/\text{kg}$). The ROC curve revealed that the cutoff point for optimal sensitivity and specificity was $50 \times 10^6/\text{L}$ for target CD34+ cells $\geq 1 \times 10^6/\text{kg}$ and $55 \times 10^6/\text{L}$ for target CD34+ cells $\geq 2 \times 10^6/\text{kg}$. The cutoff values for different target values of CD34+ cells were very close, just the HPC count difference of $5 \times 10^6/\text{L}$. So the ability of PB HPC to discriminate between PBPC yield $\geq 1 \times 10^6$ CD34+ cells/kg and 2×10^6 CD34+ cells/kg is negligible. Among those collections with CD34+ cell yield $\geq 1 \times 10^6/\text{kg}$, 75.5% had HPC level $\geq 50 \times 10^6/\text{L}$. In the present study, the positive predictive value of HPC $\geq 50 \times 10^6/\text{L}$ was 59.7% and the negative predictive value of that was 81.1% for target CD34+ cell yield $\geq 1 \times 10^6/\text{kg}$. These predictive values rely on the proportion of patients who reach the target PBPC yield: the proportion of harvests reaching the target yield of $1 \times 10^6/\text{kg}$ was 42.4%. In our series, the majority of patients had been heavily pretreated with alkylating agents, so the proportion of patients who achieved the target CD34+ cells in apheresis was small. In the previous studies, the positive predictive values of different cutoff levels of PB HPC ranging from 5 to $80 \times 10^6/\text{L}$ changed from 80% to 100% (25, 29). This is caused by the difference in the study population. In the study of Yu et al., 16.7% of the patients were healthy stem cell donors, and 80% of the collections reached the target yield of $\geq 1 \times 10^6/\text{kg}$ (29). These predictive values of HPC should be applied to a similar population of patients for which those percentages of harvests are expected to reach the target CD34+ cells.

We compared the 6 options for deciding when to commence PBPC collection to achieve CD34+ cells $\geq 1 \times 10^6/\text{kg}$. The criterion of PB HPC count $\geq 10 \times 10^6/\text{L}$ had the lowest false negative rate and that of PB WBC count $\geq 5 \times 10^9/\text{L}$ had the highest false positive rates. The PB HPC count $\geq 50 \times 10^6/\text{L}$ had better predictive values and the lowest false positive rates compared with those of the PB WBC count $\geq 5 \times 10^9/\text{L}$, $\geq 10 \times 10^9/\text{L}$ or PB HPC count $\geq 10 \times 10^6/\text{L}$, but the negative predictive value of HPC $\geq 50 \times 10^6/\text{L}$ was a little bit inferior

to that of the HPC count $\geq 10 \times 10^6/\text{L}$.

The optimal strategy to prevent unnecessary harvesting while minimizing the risk of missing an adequate harvest would be different according to the patients' premobilization characteristics. For patients who are deemed to have poor PBPC collection, such as patients who have had prior exposure to alkylating agents and radiation therapy (30, 31), the reduction of the risk of missing an adequate harvest is more important. So applying a criterion that has the lowest false negative rate, such as HPC count $\geq 10 \times 10^6/\text{L}$, would be appropriate not to miss possible adequate PBPC collection. On the other hand, for patients who seem to be good PBPC yielders, the use of a PB HPC count that had the highest positive predictive value and the lowest false positive rate, such as HPC count $\geq 50 \times 10^6/\text{L}$ would be better to avoid a harvest with poor PBPC yield.

In our study, we could not check the levels of PB CD34+ cells, which have been reported to be the best predictor of PBPC yields. So, we could not compare the clinical usefulness of PB WBC, MNC, and HPC with that of PB CD34+ cell counts to decide when to commence PBPC collection.

In conclusion, the current study suggested that the correlation between PB HPC and CD34+ cells in an apheresis product is better than the correlation between PB WBC or MNC and CD34+ cell collection. In the clinical practice setting, incorporating the data on the PB WBC, MNC, and HPC counts and applying variable cutoff values of HPC, depending on patient factors that could affect PBPC mobilization, would be a useful and rapid tool to predict the optimal timing of PBPC collection.

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