

## Original article

# A mathematical model for predicting blood volume to be processed for optimizing peripheral blood CD34+ cell yield during stem cell apheresis

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## Abstract

**Background:** Accurately predicting stem cell yields is important for planning peripheral blood stem cell (PBSC) collection.

**Objective:** This study aimed to develop a formula to forecast the blood volume required to process CD34+ cells to ensure adequate products.

**Methods:** Data from donors and patients who underwent PBSC apheresis at a tertiary care hospital were reviewed. BD FACSCanto™ II and Navios EX Flow Cytometry machines were used to determine the precollection CD34+ cell counts (pre-CD34).

**Results:** In 50 donors and 85 patients, 145 leukapheresis procedures were performed. Linear regression models accurately predicted the absolute CD34+ cell yields from pre-apheresis values, supporting the optimal planning of the procedure. Subgroup analysis identified gender, but not body weight, as a significant factor influencing collection efficiency, with lower yields in females. To ensure adequate stem cell yield, gender-specific formulas were derived to calculate the required blood volume processing. For males: Volume process (L) = (Dose CD34 [10<sup>6</sup> cells/kg] \* body weight recipient [kg] + 21) / 0.44 \* PreCD34 (cells/μL); For females: Volume process (L) = (Dose CD34 [10<sup>6</sup> cells/kg] \* body weight recipient [kg]) – 48 / 0.28 \* PreCD34 (cells/μL). Furthermore, a strong correlation ( $r = 0.981$ ) was observed between the two enumeration platforms, with Bland-Altman analysis confirming minimal bias and narrow variability. No significant variation in preCD34 was detected.

**Conclusion:** Applying preCD34 count and gender-specific predictive modeling offers valuable tools to enhance procedural efficiency and generate appropriate processed volume for the collection of adequate quantities of stem cells. Furthermore, the significance of standardizing CD34+ enumeration methods was underscored.

**Keywords:** Hematopoietic stem cell transplantation, peripheral blood stem cell apheresis, precollection CD34+ cell counts.

Hematopoietic stem cell transplantation (HSCT) is the standard of care for many malignant and non-malignant diseases. The proportion of CD34 in the HSC product collected is one of the factors crucial for successful HSCT.<sup>(1, 2)</sup> Peripheral blood stem cell (PBSC) apheresis is the preferred method to collect HSCs

rather than direct bone marrow aspiration.<sup>(3-6)</sup> A CD34+ cell count is a representative marker of hematopoietic stem cell quantity among the product obtained. The collection of CD34+ cells adequate for HSCT is crucial.

Several factors can affect the proportion of CD34+ cells in the apheresis product; these include race, ethnicity, gender, age, hematocrit, white blood cell count, the percentage of monocytes, serum ferritin, precollection CD34+ cell counts (preCD34), granulocyte colony-stimulating factor (G-CSF) dosage, the apheresis device, and technical experience. PreCD34 is the best parameter for predicting the

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adequacy of total CD34+ cells within the final product.<sup>(7)</sup> Accurately calculating blood processing volume during PBSC apheresis can help optimize leukapheresis time for individual patients, thereby reducing the workload and potential complications, such as hypocalcemia, hypotension, or thrombocytopenia during collection.<sup>(8–15)</sup>

Flow cytometry is the standardized analytical method for quantifying preCD34 and CD34 fraction within the collected hematopoietic stem cell product.<sup>(8,9)</sup> However, due to very low numbers of CD34+ cells in the bloodstream, variations in flow cytometry-based techniques can affect the accurate measurement of CD34. The factors contributing to variations in CD34 counts may be related to different reagent kits used, including the gating strategy employed for selecting the cell population analyzed.<sup>(16)</sup> Each institution has standardized and adopted protocols and procedures for these measurements.<sup>(17, 18)</sup>

This study aimed to develop mathematical models to predict the optimal blood volume to be processed based on preCD34 and patient/donor characteristics for adequate stem cell collection during PBSC apheresis. Additionally, the preCD34 counts obtained using protocols developed at a tertiary care hospital and at the National Blood Center of the Thai Red Cross Society were compared.

## Materials and methods

### Study description

This cross-sectional study analyzed allogeneic PBSC donors and patients from whom PBSCs were collected from January 2021 to January 2023. The COM.TEC blood cell separator and auto mononuclear cell (MNC) program, version 4.03.08 (Fresenius HemoCare GmbH, Bad Homburg, Germany), were used. All donors and patient demographic data were obtained. Patients had stem cells mobilized using G-CSF only or G-CSF plus chemotherapy, whereas allogeneic donors received only G-CSF as a mobilization regimen. Blood samples were collected from donors or patients undergoing allogeneic or autologous PBSC, respectively, into two ethylene diamine tetra-acetic acid tubes before starting leukapheresis, ensuring simultaneous sampling with stem cell harvesting.

### CD34+ cell enumeration

CD34+ cells were enumerated employing a single-platform ISHAGE protocol with an identical gating strategy for both flow cytometry instruments.<sup>(19)</sup> For

samples analyzed at the transfusion medicine unit of our hospital, preCD34 counts were determined utilizing the BD™ Stem Cell Enumeration Kit (BD Biosciences, San Jose, CA, USA) on a BD FACSCanto™ II flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). Samples sent to the National Blood Centre were analyzed using the Stem-Kit™ reagent on a Navios EX flow cytometer (Beckman Coulter, Brea, CA, USA). This study was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (no. 817/63).

### Statistical analysis

Data were expressed as mean, standard deviation (SD), median, and min–max range. Pearson correlation coefficients were employed to assess the degree of association between the preCD34 counts obtained employing the BD FACSCanto™ II and Navios EX Flow Cytometers. Additionally, the Bland-Altman test was applied to evaluate agreements or potential systematic differences in data obtained using the two enumeration methods, focusing on detecting bias or proportional variation in CD34 counts. A linear regression model was developed to predict the absolute CD34 yield (in number of cells) within the collected hematopoietic stem cell product from the expected CD34 yield assessed by BD FACSCanto™ II, using the following model equation,

$$\text{Absolute CD34 yield (cells)} = \text{Coefficient} * \text{Expected CD34 yield (cells)} + \text{intercept}$$

Where the coefficient represents the collection efficiency.

The expected CD34 yield (cells) was calculated as,

$$\text{Expected CD34 yield (cells)} = \text{PreCD34}(10^6 \text{ cells}/\mu\text{L}) * \text{Volume Process (L)}$$

Similarly, the absolute CD34 yield was derived utilizing the equation,

$$\text{Absolute CD34 yield (cells)} = \text{Dose CD34 (cells/kg)} * \text{body weight recipient (kg)}$$

Notably, the absolute CD34+ yield obtained from the stem cell products was used for regression analyses and measured with Navios EX. Furthermore, a multivariable linear regression model was constructed to determine the coefficient values and assess the significance of other predictors, including diagnosis, medical condition, type of HSCT, gender, and age. The R<sup>2</sup> value was calculated to evaluate the model's overall explanatory capacity and alignment with the data observed. All statistical analyses and calculations were performed using STATA version 18, with statistical significance defined as  $P < 0.05$ .

## Results

### Patient characteristics

Of the 50 healthy donors and 85 patients enrolled, 52.0% were female, and 48.0% were male. The median age was 42.0 years (range 11.0–67.0 years). The three most frequently encountered diagnoses included non-Hodgkin lymphoma (24.0%), multiple myeloma (23.0%), and acute myeloid leukemia (17.0%). Regarding the type of HSCT, autologous procedures accounted for 63.0% of cases, exceeding allogeneic transplants (37.0%), which included matched related donors and matched unrelated donors. The pre-leukapheresis complete blood count baseline is shown in **Table 1**. All patients with acute myeloid leukemia

underwent allogeneic transplants. A total of 145 leukapheresis procedures were analyzed. The median procedural time for apheresis was 292.0 min (range 130.0–410.0 min), and the median volume processed was 14.5 L (range 4.5–19.4 L).

### Precollection CD34+ cell counts

The preCD34 counts from the Navios EX and BD FACSCanto™ II platforms are shown in **Table 2**. An analysis of these preCD34 data obtained demonstrated a wide distribution across the population studied (**Figure 1**). The preCD34 counts from both instruments were positively correlated, with a prominent correlation coefficient of 0.981 (95% CI: 0.97–0.99), as shown in **Figure 2**, highlighting a consistent and substantial agreement. Furthermore,

**Table 1.** Patient characteristics (n = 135).

| Leukapheresis episode (n)         | (n) | (%) | Median  | Range          |
|-----------------------------------|-----|-----|---------|----------------|
| Age (years, median)               |     | -   | 42.0    | 11.0–67.0      |
| Body weight (kg)                  |     | -   | 60.0    | 32.9–108.7     |
| Sex                               |     |     |         |                |
| Female                            | 70  | 52  |         |                |
| Male                              | 65  | 48  |         |                |
| Diagnosis                         |     |     |         |                |
| Non-Hodgkin lymphoma              | 33  | 24  |         |                |
| Multiple myeloma                  | 31  | 23  |         |                |
| Acute myeloid leukemia            | 23  | 17  |         |                |
| Hodgkin lymphoma                  | 17  | 13  |         |                |
| Systemic sclerosis                | 5   | 4   |         |                |
| Others                            | 26  | 19  |         |                |
| Type of leukapheresis             |     |     |         |                |
| Autologous                        | 85  | 63  |         |                |
| Matched related donor (MRD)       | 29  | 21  |         |                |
| Matched unrelated donor (MUD)     | 21  | 16  |         |                |
| Hemoglobin (g/dL)                 |     |     | 10.6    | 6.9–16.5       |
| Hematocrit (%)                    |     |     | 31.9    | 19.1–49.0      |
| White Blood Cell (cells/ $\mu$ L) |     |     | 24.5    | 1.1–73.4       |
| Neutrophil (%)                    |     |     | 78.0    | 8.9–98.0       |
| Lymphocyte (%)                    |     |     | 8.0     | 1.0–97.0       |
| Monocyte (%)                      |     |     | 6.0     | 0.0–28.7       |
| Eosinophil (%)                    |     |     | 0.0     | 0.0–5.0        |
| Basophil (%)                      |     |     | 0.0     | 0.0–3.0        |
| Platelet (cells/ $\mu$ L)         |     |     | 105,000 | 37,000–307,000 |

**Table 2.** Pre-collection CD34+ cell counts.

|                                                                     | Mean | Median | SD   | Min | Max   |
|---------------------------------------------------------------------|------|--------|------|-----|-------|
| Pre-collection CD34+ cell counts, BD FACSCanto™ II (cells/ $\mu$ L) | 56.1 | 33.8   | 75.6 | 1.8 | 582.8 |
| Pre-collection CD34+ cell counts, Navios EX (cells/ $\mu$ L)        | 59.1 | 36.0   | 77.1 | 1.0 | 504.0 |

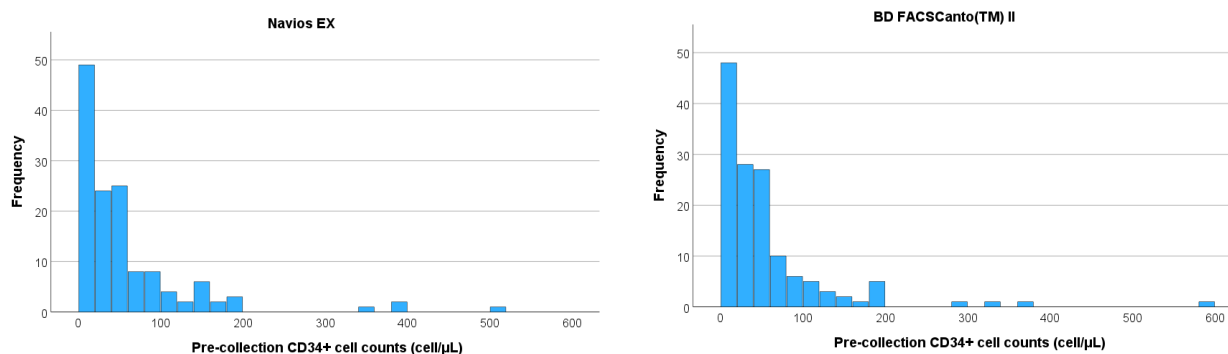


**Figure 1.** Distribution of pre-collection CD34+ cell counts from Navios EX flow cytometer (left) and BD FACSCanto™ II flow cytometer (right).

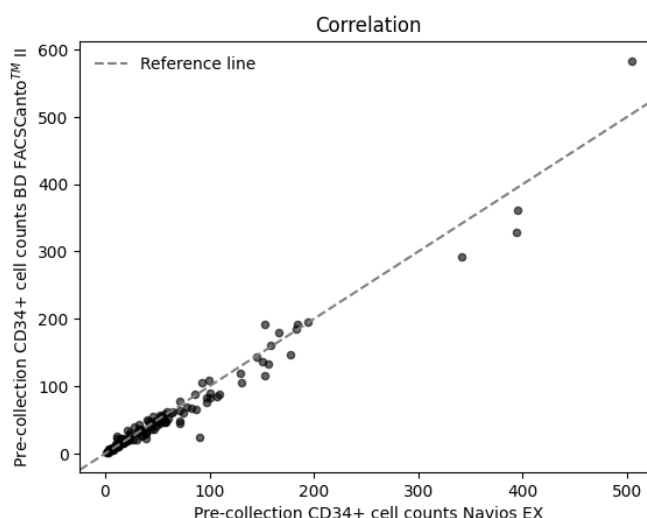


**Figure 2.** Correlation between Pre-collection CD34+ cell counts from Navios EX flow cytometer (X-axis) vs BD FACSCanto™ II flow cytometer (Y-axis).

the Wilcoxon signed-rank test produced a  $P = 0.085$ , indicating no statistically significant variations between data from the two platforms. The Bland-Altman plots further supported these findings, with a minimal median variation of 0.16 cells in preCD34 counts, and 95% of the differences falling within a narrow range of -19 to 19 cells (**Figure 3**).

The model was employed to estimate the collection efficiency coefficient at our center, which was 0.334 (**Figure 4**). It provides a quantifiable representation of the association between expected and absolute CD34 yields. It is further demonstrated by an adjusted R-squared value of 0.88, highlighting its substantial ability to explain the data obtained. The primary equation applied was,

$$\text{Absolute CD34 yield } (10^6 \text{ cells}) = 34 + (0.334 \times \text{Expected CD34 yield } [10^6 \text{ cells}])$$

#### **Analysis of factors associated with absolute CD34+ yield**

Subgroup analysis investigated potential factors influencing absolute CD34+ yield, including gender, age, donor body weight, type of HSCT, and medical conditions diagnosed. Preliminary examinations identified a significant association between gender and expected CD34 yield (**Figure 5**). To account for the gender-specific differences in collection efficiency observed, an interaction term (sex, donor, or expected CD34 yield) was included within the multivariable model to evaluate whether the impact of expected on absolute CD34 yield was modified by gender. Employing these identified factors, we further determined the collection efficiency coefficient *via* a multiple linear regression model using the equation,

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3 * (X_1 * X_2)$$

Where:

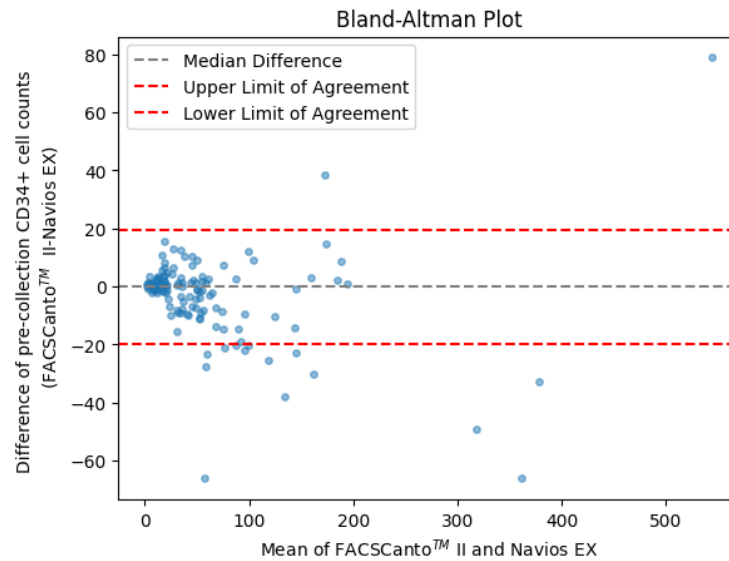


**Figure 3.** Bland-Altman plot.

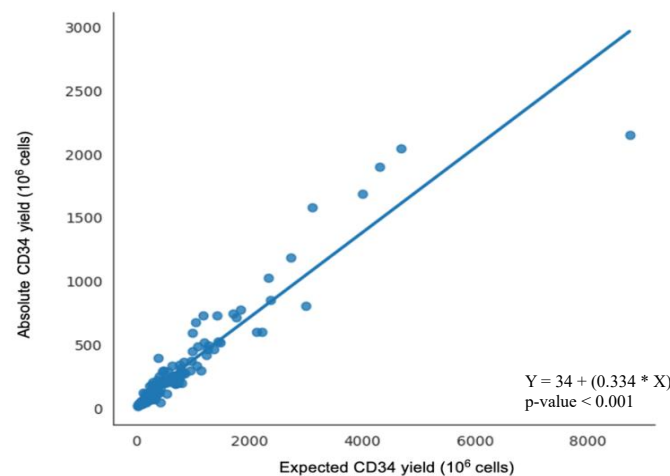


**Figure 4.** Linear regression of absolute CD34 yield and expected CD34 yield with residual plot.

- $Y$  represents the absolute CD34 yield.
- $X1$  is the expected CD34 yield.
- $X2$  denotes sex (male = 1, female = 0).
- $b3$  captures the interaction between sex and expected CD34 yield on the absolute CD34 yield.

The results of uni and multivariable linear regression analyses for factors associated with absolute CD34+ yield are summarized in **Table 3**.

Introduction of the “interaction” term substantially improved the model fit ( $P < 0.001$ ), underscoring its significance in accounting for variance in the absolute CD34 yield within the final product. The final regression model was expressed as,

$$\text{Absolute CD34 yield (10}^6 \text{ cells)} = 48 + (0.28 * \text{Expected CD34 yield [10}^6 \text{ cells]}) - (69 * \text{Sex}) + (0.16 * \text{Sex} * \text{Expected CD34 yield [10}^6 \text{ cells]})$$

This equation can be simplified for each sex as,

$$\text{For males: Absolute CD34 yield (10}^6 \text{ cells)} = (0.44 * \text{Expected CD34 yield [10}^6 \text{ cells]}) - 21$$

$$\text{For females: Absolute CD34 yield (10}^6 \text{ cells)} = (0.28 * \text{Expected CD34 yield [10}^6 \text{ cells]}) + 48$$

To further operationalize this model in clinical practice, we used the relationship,

$$\text{Expected CD34 yield (10}^6 \text{ cells)} = \text{PreCD34 (cells/}\mu\text{L)} \times \text{Volume Processed (L)}$$

This equation allows estimation of the total expected CD34 yield based on the donor’s preCD34 count and apheresis volume. Substituting these values into the above equations, we derived formulas to estimate the total blood processing volume (L) needed to achieve a target CD34+ dose (Dose CD34 [cells/kg]), factoring in recipient body weight and donor characteristics:

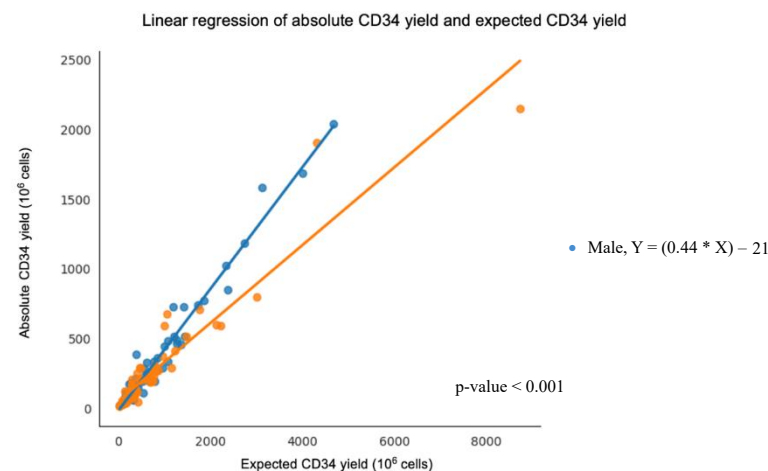


Figure 5. Subgroup analysis plot by donor sex.

Table 3. Factors associated with absolute CD34+ yield.

|                                    | Univariate analysis    |                         |         | Multivariate analysis |                         |         |
|------------------------------------|------------------------|-------------------------|---------|-----------------------|-------------------------|---------|
|                                    | Unadjusted coefficient | 95% confidence interval | P-value | Adjusted coefficient  | 95% confidence interval | P-value |
| Expected CD34 yield                | 0.34                   | 0.32 to 0.36            | <0.001  | 0.28                  | 0.26 to 0.30            | <0.001  |
| Sex donor                          |                        |                         |         |                       |                         |         |
| Female                             | Baseline               |                         |         |                       |                         |         |
| Male                               | 91.09                  | -37.97 to 220.15        | 0.165   | -93.80                | -140.78 to 46.81        | <0.001  |
| Type                               |                        |                         |         |                       |                         |         |
| Autologous                         | Baseline               |                         |         |                       |                         |         |
| MRD                                | -31.34                 | -194.08 to 131.39       | 0.704   | -39.98                | -82.28 to 2.31          | 0.064   |
| MUD                                | 29.97                  | -154.43 to 214.37       | 0.748   | -25.54                | -75.45 to 24.38         | 0.313   |
| Donor age (years)                  | 3.65                   | -0.92 to 8.21           | 0.117   | 0.70                  | -0.56 to 1.95           | 0.273   |
| Donor body weight (kg)             | 3.80                   | -0.36 to 7.96           | 0.073   | 1.16                  | -0.10 to 2.42           | 0.071   |
| [Sex donor] x [Expected CD4 yield] |                        |                         |         | 0.16                  | 0.13 to 0.19            | <0.001  |

MRD, matched related donor, MUD, matched unrelated donor.

- For males  
 $Volume\ process\ (L) = (Dose\ CD34\ [10^6\ cells/kg] * body\ weight\ recipient\ [kg] + 21)/0.44 * PreCD34\ (cells/\mu L)$
  - For females  
 $Volume\ process\ (L) = (Dose\ CD34\ [10^6\ cells/kg] * body\ weight\ recipient\ [kg] - 48)/0.28 * PreCD34\ (cells/\mu L)$
- Example:

To achieve a target dose of  $5 \times 10^6$  CD34+ cells/kg for a 50 kg recipient with a PreCD34 of 100 cells/ $\mu$ L, the equations applied were,

- Males:**  
 $Volume\ to\ process = ([5 \times 50] + 21)/(0.44 \times 100) = 6.16\ L$
- Females:**  
 $Volume\ to\ process = ([5 \times 50] - 48)/(0.28 \times 100) = 7.21\ L$

## Discussion

We successfully established an equation for predicting the target CD34+ dose within the apheresis product based on preCD34 and gender using data from the Thai population. The linear regression model developed not only predicted absolute CD34 yield based on pre-apheresis values but also provided practical guidance for determining the total processed blood volume and procedure duration required. Such a capability reduces procedural variability and enhances resource utilization, aligning with recommendations from studies advocating predictive modeling to improve stem cell collection efficiency.

A notable finding is the impact of donor gender on collection efficiency. Female donors had significantly lower collection efficiency coefficients, as shown by the subgroup analyses. This observation aligns with studies that identified “female gender” as a negative predictor of CD34 yield, potentially due to differences in baseline hematopoietic activity and mobilization response.<sup>(20–22)</sup> Under similar conditions, female donors require a larger apheresis volume than males to achieve the same target CD34+ dose, highlighting the prominent impact of gender on collection efficiency and the importance of personalized collection strategies. Importantly, this disparity in collection efficiency was not attributable to variations in donor body weight, as it did not emerge as a significant factor influencing preCD34 counts. Our formula was confirmed as a precise approach for predicting the blood volume to be processed required for CD34+ collection. Alternatively, Gilcher’s formula addressed gender-related inequities by applying different equations for calculating total blood volume, which used varied constants for males and females.

Our findings suggest that biological or hormonal factors may play a more prominent role than physical attributes such as weight in determining mobilization efficacy. The multivariable regression model developed in this study incorporated gender as a predictor, further underscoring the need for tailored apheresis strategies that account for the characteristics of individual donors. The predictive models exhibited strong explanatory power, as indicated by the high adjusted R-squared value (0.88). By incorporating interaction terms, the models effectively accounted for the combined impacts of gender and preCD34 on collection outcomes.

Using the mathematical formulas described provided actionable insights for clinicians to customize apheresis protocols and more efficiently achieve target CD34 doses. Such precision is particularly critical in HSCT, where reaching optimal CD34 doses is associated with improved engraftment success and patient outcomes.<sup>(2,9)</sup> Practically, the apheresis time in a few patients with elevated preCD34 levels could be reduced compared with an approach where the fixed processing blood volume is three times the patient’s estimated blood volume. Our formula can also be applied to predict the number of days needed for leukapheresis and determine plerixafor usage to help salvage failed mobilizations. This strategy may help reduce apheresis-associated adverse effects, improve patient satisfaction, and maximize resources such as apheresis time, the requirement for a technician, and the necessary space.

The consistency between preCD34 counts obtained from the BD FACSCanto™ II and Navios EX flow cytometers highlighted the interchangeability of these platforms for clinical use. The robust correlation and minimal bias reported by the Bland-Altman analysis confirmed the reliability of the two instruments. Critically, the absence of statistically significant variations in CD34 enumerations underscores their comparability and supports the feasibility of harmonizing protocols across institutions. These findings are consistent with research emphasizing standardization in flow cytometric techniques for hematopoietic stem cell enumeration.<sup>(18)</sup> The determination of a collection efficiency coefficient specific to our hospital also represents a significant step toward procedurally optimizing leukapheresis.

The study also underscores the importance of external quality assessment programs in ensuring the reliability of flow cytometry data. The Navios EX platform, utilized by the National Blood Center, adheres to ISO standards and participates in UK NEQAS proficiency testing. Such a rigorous quality assurance framework contributes to the reproducibility and validity of CD34 enumerations. The value of standardized protocols and external benchmarking in achieving consistent results across institutions has been emphasized.<sup>(17, 18)</sup>

While the findings of this report are robust, some limitations warrant consideration. First, the study population predominantly consisted of patients undergoing autologous transplantation, limiting the generalizability of data to allogeneic settings. Second,

the collection efficiency for each model or machine is different. Therefore, this formula may not apply to other apheresis machines. Third, this was an *in vitro* study, and further clinical evaluations, such as engraftment rate, are warranted to validate this gender-based formula. Additionally, the influence of mobilization regimens on absolute CD34 yield was not extensively explored. Future research must focus on expanding the scope of predictive models and validating them across diverse populations and clinical settings to further refine HSCT protocols. Furthermore, incorporating additional flow cytometry platforms and evaluating their performance under varied clinical scenarios could enhance the applicability of these findings.

## Conclusion

Using the model formula developed, the total processed blood volume required for leukapheresis can be accurately calculated. By addressing donor-specific factors, such as gender, these findings pave the way for a more personalized approach to stem cell collection. This ability could help decide the use of novel mobilization agents in some patients and lower the adverse effects associated with PBSC apheresis. This study also underscored the critical role of standardized CD34 enumeration and predictive modeling in optimizing apheresis procedures for HSCT. The agreement between BD FACSCanto™ II and Navios EX platforms, combined with the development of institution-specific efficiency coefficients, offers a practical framework for improving procedural capability and patient outcomes.

## Author contributions

RV contributed substantially to the concept and design of this study, acquiring data, reviewing literature, and its analysis and interpretation. KS performed the experiments and collected the data. KC conducted the experiments. PW and RV contributed to drafting the manuscript. PW and PR edited the manuscript critically for scientific content. All authors approved the version submitted and take responsibility for statements made in the published article.

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## Conflict of interest statement

The authors declare no conflict of interest.

## Data sharing statement

Data supporting the findings of this study are available from the corresponding author and can be provided upon reasonable request.

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