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Peripheral Blood Stem Cell Harvest HPC Count Is an Effective Surrogate Marker for CD34+ Cell Count in Allogeneic Stem Cell Transplant Setting

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ABSTRACT

OBJECTIVE: We assessed the predictive potential of XN-HPC for CD34+ cell count as obtained through Sysmex automated hematology analyzers (XN-1000). METHODS: This study was conducted at the National Institute of Blood Diseases and Bone Marrow Transplantation in 84 donors between December 2012 and December 2017 in the first phase and later validated in 112 donors between December 2017 and December 2018. Sysmex XN-1000 and BD FACS Calibur estimated XN-HPC and CD34+ cells of peripheral blood apheresis product, respectively. Spearman's correlation was assessed between XN-HPC and CD34+ cell count followed by receiver operating characteristic curve calculation to determine the XN-HPC cutoff for a CD34+ count of ≥2 million cells/kg of recipient's body weight RESULTS: There is a moderately positive correlation (*P* value = .003) between XN-HPC and CD34+ count. Receiver operating characteristic curve analyses demonstrated that a cutoff value for XN-HPC of $\geq 1{\cdot}845 \times 10^6$cells/kg of recipient's body weight has a specificity and sensitivity of 100% and 78·2%, respectively, for predicting the CD34+ count of ≥2 million cells/kg of recipient's body weight. This cutoff value of XN-HPC was prospectively validated in 112 donors. The positive predictive value was found to be 100%, while negative predictive value was 17%. CONCLUSION: XN-HPC has a highly promising potential to serve as a cost-effective and time-saving surrogate for CD34+ cell count.

Introduction

Allogeneic hematopoietic stem cell transplant provides the only chance of radical cure for various life-threatening benign and malignant hematological disorders [1–3]. A successful bone marrow transplant is one in which complete sustained hematopoietic reconstitution is achieved. Success relies chiefly on the infusion of appropriate dose of hematopoietic progenitor cells (HPCs), i.e., ≥ 2million CD34+cells/kg of recipient's body weight, and their engraftment, repopulating the hematopoietic tissue [4–8].

Colony-forming unit–granulocyte monocyte (CFU-GM) assay was considered to be the best established predictor of progenitor cell count [9–12]. Over the years, reliable enumeration of CD34+ cell count by flow cytometry has efficiently replaced CFU-GM assay, and it is comprehensively accepted to be a reliable indicator of hematopoietic progenitor cells [13–21]. However, the enumeration of CD34+cells by flow cytometry is not only an expensive procedure [22]; it is also time consuming (with a response time up to several hours) and requires substantial technical expertise which in itself is a significant challenge in low- and middle-income countries [23,24]. Flow cytometry–based Sysmex automated hematology analyzers segregate a distinct immature myeloid population of cells referred to as hematopoietic progenitor cells (XN-HPC) [25,26]. The analyzer is equipped with a white precursor cell (WPC) channel (Sysmex XN-1000). It processes the cells with a distinct reagent system. The surfactant-detergent in the reagent system lyses the mature myeloid cells, but the

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immature myeloid cells escape lysis due to the lower cholesterol content of their cell membranes. Analysis of immature cells is then carried out by employing optical detection and general flow cytometry in XN-1000 [27,28]. The technique does not demand pretreatment of blood samples, nor does it require any distinct expertise, and it has a maximum turnaround time of 90 seconds.

Various studies have elucidated the role of peripheral blood HPC count as a predictor of apheresis day to yield an optimum value of CD34+ cells in autologous stem cell transplant setting. Detection of HPC has been greatly improved in new generation of Sysmex XN series, and incipient statistics are suggestive of good correlation between XN-HPC and CD34+ cells assessment by flow cytometry [21–26]. The current study aims to establish the possible role of postharvest product XN-HPC count as a reliable surrogate of postharvest product CD34+ cell count in allogeneic bone marrow transplant setting.

Methods

Study Design and Setting

This two-phase study was aimed at determining the optimal cutoff value of XN-HPC count to predict CD34+ cell count of ≥2 million cells/kg of recipient's body weight, followed by the prospective validation of the established XN-HPC cutoff. The study was conducted at the National Institute of Blood Disease & Bone Marrow Transplant (NIBD & BMT) from December 2012 till December 2018 after approval from the Ethics Review Committee of NIBD & BMT.

In the first phase conducted between December 2012 and December 2016, all patients and their human leukocyte antigen (HLA)–matched donors undergoing allogenic hematopoietic stem cell transplant were enrolled after informed consent and assessed for XN-HPC count and CD34 cell count to determine optimal cutoff value.

In the second phase, conducted from January 2017 till December 2018, 112 donors were prospectively enrolled to validate the cutoff value.

Patients and Donors

Patients with various benign and malignant hematological disorders, and their respective HLA-matched donors were recruited in the study after obtaining informed written consent by the donors and patients. In case of donors/patients aged less than 18 years, parental assent was ensured. Inclusion criterion for donor comprised of age between 6 and 55 years and satisfactory hepatic, renal, pulmonary, and cardiac reserves.

Priming of Bone Marrow and Mobilization of Peripheral Blood Stem Cells

Healthy HLA-matched donors (siblings or parents) were treated with injection of granulocyte–colony-stimulating factor at 10 mg/kg body weight subcutaneously for 4 days to mobilize stem cells. Peripheral blood apheresis was performed on day five. Seventeen (20%) donors underwent two sessions of peripheral blood apheresis to achieve the minimum target of $\geq 2 \times 10^6$ CD34+ cells/kg of recipient's body weight. All the priming and mobilization protocols along with the stem cell collection and infusion procedures were preapproved by the institutional review board of NIBD and BMT.

Stem Cell Collection

Peripheral Blood Hematopoietic Stem Cell Harvesting

Peripheral blood apheresis was performed by employing COBE SPECTRA or Hemoneitics MCS plus cell separators processing 1.5-2 volumes of donor's blood per session over a 3- to 5-hour period. Repeat apheresis session was done on day six (second collection) if the desired target of CD34+ cell count of $\geq 2 \times 10^6$ cells/kg of recipient's body weight was not attained in the first session. Sodium citrate to blood ratio of 1:9 was set for anticoagulation.

Enumeration of HPC count

Immature myeloid cells (HPCs) of the peripheral blood apheresis product were enumerated by Sysmex XN-1000. The analyzer utilizes a refined technology of fluorescence flow cytometry for enumerating HPC count. After processing the cells with the surfactant-detergent system in its WPC and pathologic cell channel, it produces forward scatter (FSC), side scatter (SSC), and fluorescence scatter of cell populations. The FSC and SSC give information about the cell size and its internal complexity, respectively. The fluorescence intensity (Side Fluorescence) generates information about the maturity status of cells and its benign/malignant origin. Accredited to their lower membrane lipid content, juvenile myeloid cells have lower WPC reagent permeability. Hence, the HPCs are recognized by their medium FSC, low SSC, and relatively low Side Fluorescence. The values of HPC as obtained in $10^3/\mu l$ were converted to and represented as cells/kg of recipient's body weight to make the two variables, i.e., CD34+ cells and HPC, comparable.

Enumeration of CD34+ Cells by Flow Cytometry

BD FACS Calibur was commissioned to enumerate CD34+ cell count by flow cytometry of apheresis product based on highly sensitive and specific gold standard International Society for Hematotherapy and Graft Engineering gating strategy [29]. The fluorochrome antibody panel comprised of CD38-FITC, HLA-DR-PE, and CD34-APC. One million cells were stained for each of the three tubes being analyzed. After RBCs lysis with standard RBC lysing solution, 20 μl of CD38-FITC and HLA-DR-PE was added in tube 1 and 2, and 5 μl of CD34-APC was added in tube 3. The tubes with their respective fluorochromes were incubated in the dark for 30minutes and then were suspended in 1% formaldehyde solution. This was then followed by acquisition of data by flow cytometer and analysis by the CELLQUEST Pro software. The computed result for CD34+ cells was represented as cells $\times 10^6$ per kilogram of recipient's bodyweight.

Statistical Analysis

Statistical analyses were performed using STATA version 11. Median and range were reported for continuous variables as the data were not normally distributed, whereas frequencies and percentages mwere reported for all categorical variables. Spearman's rank correlation coefficient (ρ) was calculated for CD34+ cells and HPC, followed by the calculation of receiver operating characteristic (ROC) curve to identify HPC value, which could optimally distinguish the cutoff of ≥2 million or more for CD34+ cells/kg of recipient's body weight. CD34+ cell count obtained in the first peripheral blood apheresis session was used as the predicted variable in case donor underwent more than one session. *P* values less than or equal to .05 were considered statistically significant.

Results

Phase I Results

Donor Characteristics

There were a total of 84 donors with a median age of 20 years (range 6-51 years) and comprised of 51 (61%) females.

HPC Count and CD34 Count in the Postharvest Products

A total of 101 peripheral blood-harvesting procedures after GCSF priming for 4 days were undertaken. Mean volume of the postharvest product collected was 257 (±139) ml. The median XN-HPC and CD34+ counts were 3.83 (range 0.32-20.00) × 10^6cells/kg of the recipient's body weight and 4.55 (range 1.00-26.20) × 10^6cells/kg of the recipient's body weight, respectively.

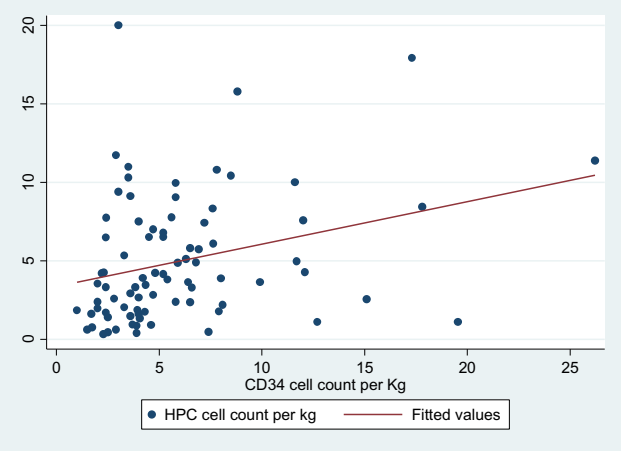

Figure 1. Scatter plot showing distribution of HPC and CD34+ × 10^6 cells/kg.

Correlation Between CD34 Cell Count and HPC Count

Spearman's correlation analysis showed a $\rho = 0.32$ (P value = ·003), indicating a moderately positive and statistically significant correlation between CD34+ cell count and HPC count as graphically shown in Figure 1 scatter plot.

Sensitivity and Specificity of Postharvest HPC as Predictor of Postharvest CD34

The CD34 cutoff value of ≥2 × 10^6 CD34+ cells/kg of the recipient's body weight was used as target value to evaluate corresponding HPC count. ROC curve analysis (Figure 2) showed area under the curve as 87.0% (P value = .013).

Multiple XN-HPC cutoff values were computed for the CD34 value of ≥2 × 10^6 CD34+ cells/kg to establish evaluable sensitivity and specificity of XN-HPC for a postharvest hematopoietic stem cell competent product, i.e., the product with the CD34+ cell count of ≥2 × CD34+ × 10^6 cells/kg of the recipient's body weight as shown in Table 1.

An XN-HPC cutoff value of ≥1.84 × 10^6 cells/kg of the recipient's body weight showed sensitivity and specificity of 78.2% and 100.0%, respectively, implicating that if this XN-HPC cutoff is used to predict CD34+ cell count, all donors will have a CD34+ count of ≥2 × 10^6 cells/kg. Sixty-one (74.4%) of the evaluable donors had XN-HPC values ≥1·84 million cells/kg of the recipient's body weight, and all of them were found to have the flow cytometry–based CD34+ cell count of ≥2 × 10^6 CD34+ cells/kg of the recipient's body weight.

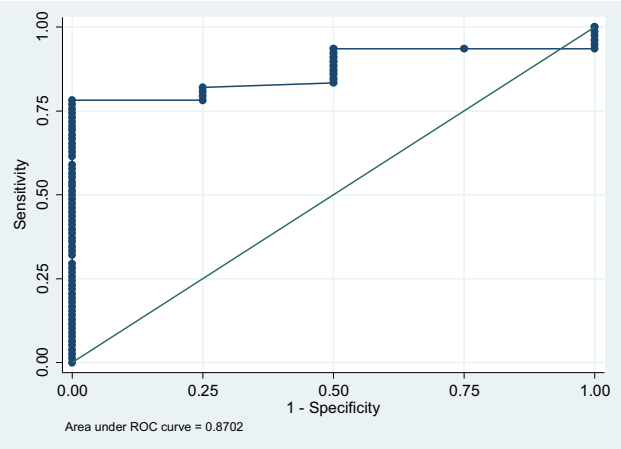

Figure 2. Receiver operating characteristic curve.

Table 1
Comparison of Sensitivities and Specificities at Different Cutoff Levels of HPC Cells Based on ROC Curve

Coordinates of the Curve		
Test result variable(s): HPC (10^6 cell/kg)		
Positive if greater than or equal to	Sensitivity (%)	Specificity (%)
.358000	98.70	.00
.685000	93.60	25.00
.805000	93.60	50.00
1.660000	82.10	75.00
1.715000	80.80	75.00
1.745000	79.50	75.00
1.795000	78.20	75.00
≥1.845000	≤78.20	100.00

Phase II Results

Validation of XN-HPC Cutoff Value

One hundred and twelve donors were enrolled for validation study. The positive predictive value was found to be 100%, while the negative predictive value was 17%. The median and range of XN-HPC count were found to be 4.49 (0.32-95.20) million cells per kg of recipient's body weight, whereas the median and range CD34 cell count were 4.7 (1.00-61.00) million per kg of recipient's body weight. Similarly, in order to predict CD34+ cell count of >3 × 10^6 cells/kg of the recipient's body weight, the corresponding XN-HPC count was found to be 13.765 million cells/kg of the recipient's body weight in the first phase. It was also prospectively validated and conferred to the specificity of positive predictive value of 100%. However, we were not able to identify any HPC cutoff to predict CD34+ cell count of 4 million or above due to the limitation of small data set.

Discussion

The success of allogeneic hematopoietic stem cell transplant depends majorly on the successful repopulation of hematopoietic tissue after infusion of an adequate dose of hematopoietic stem cells. Over the years, different modalities have been employed to prognosticate the dose of stem cells in a postharvest product such as CFU-GM assays, total nucleated cell counts, and CD34+ cell count.

This study attempted to evaluate the role of postharvest product XN-HPC as a potential surrogate of postharvest product CD34+ cell count, which is the current worldwide standard for evaluating the stem cell competency of the collected hematopoietic stem cell products, in the allogeneic stem cell transplant setting.

The need to establish this correlation between postharvest XN-HPC and CD34+ cells stems from the fact that, in resource-poor countries, estimation of CD34+ cell count by flow cytometry is significantly challenging in terms of cost, the technical expertise it requires, and its turnaround time. On the other hand, XN-HPC is an extremely cost-effective procedure requiring no special expertise and has a turnaround time of over a few seconds.

In autologous stem cell transplant setting, preharvest peripheral blood HPC has already been established as a successful determinant of the apheresis day for stem cell collection for effective product collection [30]. Another recent study has successfully established the comparable predictive potential of preharvest peripheral blood XN-HPC to postharvest product CD34 count in both the autologous and allogeneic stem cell transplant settings [4].

In this study, XN-HPC by Sysmex automated analyzer and CD34 count by flow cytometry were performed on 101 postharvest samples as collected from 84 donors for allogeneic stem cell transplant. As of the writing of this paper and to the best of our knowledge, no study has yet evaluated the predictive potential of postharvest product XN-HPC for postharvest product CD34+ cells in a majorly allogeneic setting.

The results obtained have successfully elaborated the high predictive potential of XN-HPC as a surrogate marker of post-harvest stem cell product competency. The CD34+ cells cutoff value of $\geq 2 \times 10^6$CD34+ cells/kg of the recipient's body weight is the comprehensively accepted value that ascertains successful posttransplant hematopoietic tissue repopulation. The results demonstrated that the XN-HPC value of $\geq 1{\cdot}84 \times 10^6$ cells/kg of recipient's body weight carries the sensitivity and specificity of 78.2% and 100.0%, respectively. This implicates that, in our study, the HPC cutoff value of ≥1.84 million cells/kg of recipients' body weight successfully predicted the competence of stem cell harvest product, i.e., the presence of $\geq 2 \times 10^6$ CD34+ cells/kg of the recipient's body weight, in 100% of the harvested products analyzed. Spearman's correlation analysis showed a $\rho = 0.32$ (P value = .003), demonstrating a statistically significant correlation between CD34+ cell count and XN-HPC count in postharvest stem cell product. The results were prospectively validated over a period of 1 year in 112 donors, yielding a positive and negative predictive value of 100% and 17%, respectively.

Conclusion

The findings concluded that there exists a fairly high potential for XN-HPC to serve as a surrogate for CD34+ cell count. The findings demand larger multicentric studies to further validate the results to establish XN-HPC as a preferred cost-effective and time-efficient method of establishing stem cell competency of postharvest products in resource-poor countries.

Declarations

Competing Interests

The authors declare that they have no competing interests.

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Data Statement

The research data are confidential.

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References

[1] H.K. Mahmoud, A.M. Elhaddad, O.A. Fahmy, Samra M.A. Abdelfattah RM, Y.H. El-Nahass, et al., Allogeneic hematopoietic stem cell transplantation for non-malignant hematological disorders, J. Adv. Res. 6 (2015) 449–458, https://doi.org/10.1016/j.jare.2014.11.001.

[2] A. Gratwohl, H. Baldomero, J.F. Passweg, F. Frassoni, D. Niederwieser, N. Schmitz, et al., Hematopoietic stem cell transplantation for hematological malignancies in Euruope, Leukemia 17 (5) (2003) 941–959, https://doi.org/10.1038/sj.leu.2402896.

[3] E.D. Thomas, Bone marrow transplantation: a review, Sem in Hematol 36 (4 Suppl 7) (1999) 95–10310595758.

[4] E.I. Peerschke, C. Moung, M.S. Pessin, P. Maslak, Evaluation of new automated hematopoietic progenitor cell analysis in the clinical management of peripheral blood stem cell collections, Transf 55 (8) (2015) 2001–2009, https://doi.org/10.1111/trf.13078.

[5] J.G. Bender, To LB, S. Williams, L.S. Schwartzberg, Defining a therapeutic dose of peripheral blood stem cells, J. Hematother. 1 (4) (1992) 329–341, https://doi.org/10.1089/scd.1.1992.1.329.

[6] O. Hequet, Hematopoietic stem and progenitor cell harvesting: technical advances and clinical utility, J Blood Med 18 (6) (2015) 55–67, https://doi.org/10.2147/JBM.S52783.

[7] To LB, D.N. Haylock, P.J. Simmons, C.A. Juttner, The biology and clinical uses of blood stem cells, Blood 89 (7) (1997) 2233–22589116266.

[8] J. D'Rozario, R. Parisotto, J. Stapleton, A. Gidley, D. Owen, Pre infusion, post thaw CD34+ peripheral blood stem cell enumeration as a predictor of haematopoietic engraftment in autologous haematopoietic cell transplantation, Transf Apher Sci 50 (3) (2014) 443–450, https://doi.org/10.1016/j.transci.2014.02.021.

[9] L. Schwartzberg, R. Birch, R. Blanco, Wittlin F, Muscato J, Tauer K. Rapid and sustained hematopoietic reconstitution by peripheral blood stem cell infusion alone following high-dose chemotherapy, Bone Marrow Transplant. 11 (5) (1993) 369–3748099300.

[10] L. Douay, N.C. Gorin, J.Y. Mary, E. Lemarie, M. Lopez, A. Najman, et al., Recovery of CFU-GM from cryopreserved marrow and in vivo evaluation after autologous bone marrow transplantation are predictive of engraftment, Exp. Hematol. 14 (5) (1986) 358–3653519263.

[11] A.D. Elias, L. Ayash, K.C. Anderson, M. Hunt, C. Wheeler, G. Schwartz, et al., Mobilization of peripheral blood progenitor cells by chemotherapy and granulocyte-macrophage colony-stimulating factor for hematologic support after high-dose intensification for breast cancer, Blood 79 (11) (1992) 3036–30441350229.

[12] C.H. Weaver, B. Hazelton, R. Birch, P. Palmer, C. Allen, L. Schwartzberg, et al., An analysis of engraftment kinetics as a function of the CD34 content of peripheral blood progenitor cell collections in 692 patients after the administration of myeloablative chemotherapy, Blood 86 (10) (1995) 3961–39697579367.

[13] I. ChinYee, L. Anderson, M. Keeney, D.R. Sutherland, Quality assurance of stem cell enumeration by flow cytometry. Cytometry, J Int Soc for Analyt Cytol. 30 (6) (1997) 296–3039440821.

[14] G. Fritsch, D. Printz, M. Stimpfl, M.N. Dworzak, V. Witt, U. Pötschger, et al., Quantification of CD34+ cells: comparison of methods, Transf. 37 (8) (1997) 775–784, https://doi.org/10.1046/j.1537-2995.1997.37897424398.x.

[15] J.W. Gratama, A. Orfao, D. Barnett, B. Brando, A. Huber, G. Janossy, et al., Flow cytometric enumeration of CD34+ hematopoietic stem and progenitor cells, Cytometry 34 (3) (1998) 128–1429696157.

[16] C. Elliott, D.M. Samson, S. Armitage, M.P. Lyttelton, D. Mc Guigan, R. Hargreaves, et al., When to harvest peripheral-blood stem cells after mobilization therapy: prediction of CD34-positive cell yield by preceding day CD34-positive concentration in peripheral blood, J. Clin. Oncol. 14 (3) (1996) 970–973, https://doi.org/10.1200/JCO.1996.14.3.970.

[17] I. Fatorova, M. Blaha, M. Lanska, D. Vokurkova, V. Rezacova, P. Zak, Timing of peripheral blood stem cell yield: comparison of alternative methods with the classic method for CD34 cell determination, Bio Med Res Int. (2014) 1–13, https://doi.org/10.1155/2014/575368.

[18] A. Kikuchi-Taura, T. Soma, T. Matsuyama, D.M. Stern, A. Taguchi, A new protocol for quantifying CD34+ cells in peripheral blood of patients with cardiovascular disease, Tex. Heart Inst. J. 33 (4) (2006) 427–42917215964.

[19] M. Keeney, I. Chin-Yee, K. Weir, J. Popma, R. Nayar, D.R. Sutherland, Single platform flow cytometric absolute CD34+ cell counts based on the ISHAGE guidelines, Cytometry: J Int Soc Analyt Cytol. 34 (2) (1998 Apr 15) 61–709579602.

[20] D.R. Sutherland, A.K. Stewart, A. Keating, CD34 antigen: molecular features and potential clinical applications, Stem Cells 11 (S3) (1993) 50–57, https://doi.org/10.1002/stem.5530110914.

[21] Sutherland DR, Keeney M. Gratama JW. Enumeration of CD34+ hematopoietic stem and progenitor cells. Curr Prot Cytom 2003;25(1):6–4. https://doi.org/10.1002/0471142956.cy0604s25

[22] R. Letestu, C. Marzac, F. Audat, R. Belhocine, S. Tondeur, V. Baccini, et al., Use of hematopoietic progenitor cell count on the Sysmex XE-2100 for peripheral blood stem cell harvest monitoring, Leukemia & lymphoma. 48 (1) (2007) 89–96, https://doi.org/10.1080/10428190600886149.

[23] Z. Zulkafli, R. Mustaffa, S.M. Yusoff, Hematopoietic progenitor cells as a predictive of CD34+enumeration prior to peripheral blood stem cells harvesting, Bali Med J 3 (3) (2014) 112–115, https://doi.org/10.15562/bmj.v3i3.85.

[24] U. Oelschlaegel, M. Bornhaeuser, C. Thiede, G. Ehninger, K. Hoelig, HPC enumeration with the Sysmex XE-2100 can guide further flow cytometric CD34+ measurements and timing of leukaphereses, Cytotherapy 5 (5) (2003) 414–419, https://doi.org/10.1080/14653240310003071.

[25] K.U. Park, S.H. Kim, C. Suh, S. Kim, S.J. Lee, J.S. Park, et al., Correlation of hematopoietic progenitor cell count determined by the SE-9000™ automated hematology analyzer with CD34+ cell count by flow cytometry in leukapheresis products, Am. J. Hematol. 67 (1) (2001) 42–47, https://doi.org/10.1002/ajh.1074.

[26] B.W. Steussy, M. Capper, M.D. Krasowski, N.S. Rosenthal, A.J. Schlueter, Algorithms utilizing peripheral blood hematopoietic progenitor cell counts in lieu of some CD34+ cell counts predict successful peripheral blood stem cell collections with substantial time and cost savings, ISBT Sci Ser 11 (3) (2016) 153–162, https://doi.org/10.1111/voxs.12289.

[27] T. Yamane, K. Takekawa, N. Tatsumi, Possibility of identification of hematopoietic stem cells using a conventional blood cell counter, Eur. J. Haematol. 55 (3) (1995) 207–208, https://doi.org/10.1111/j.1600-0609.1995.tb00254.x.

[28] Takekawa K, YamaneT,Suzuki K, Hino M, Tatsumi N. Identification of hematopoietic stem cells by the SE-9000™ automated hematology analyzer in peripheral blood stem cell harvest samples. Acta Haematol. 1997;98:54–5. https://doi.org/10.1159/000203564

[29] D.R. Sutherland, L. Anderson, M. Keeney, R. Nayar, I.A. Chin-Yee, The ISHAGE guidelines for CD34+ cell determination by flow cytometry, J. Hematother. 5 (3) (1996) 213–226, https://doi.org/10.1089/scd.1.1996.5.213.

[30] F. Lefrère, S. Zohar, S. Beaudier, F. Audat, J.A. Ribeil, D. Ghez, et al., Evaluation of an algorithm based on peripheral blood hematopoietic progenitor cell and CD34+ cell concentrations to optimize peripheral blood progenitor cell collection by apheresis, Transf 47 (10) (2007) 1851–1857, https://doi.org/10.1111/j.1537-2995.2007.01407.x.