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RESEARCH ARTICLE

Systematic Investigation of the Location of CD34+ Cells Within the Mononuclear Cell Layer of Hematopoietic Progenitor Cell Collections in Normal Donors

Minlin Wu | Stephanie Dormesy | Patricia A. Shi

New York Blood Center, Rye, New York, USA

Correspondence: Patricia A. Shi (pshi@nybc.org)

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ABSTRACT

With hematopoietic progenitor cell (HPC) collections by apheresis, it is not clear whether CD34+ cells inhabit a specific density within the mononuclear cell (MNC) interface. This study was undertaken to see whether this could be determined by targeting a specific depth in the interface as determined by the colorgram level. In 90 National Marrow Donor Program HPC collections using the continuous MNC procedure of the Spectra Optia device, the level of the MNC layer targeted for collection was systematically rotated between three different colorgram levels. Relevant collection characteristics, collection efficiencies (CE), and a novel measure, the CD34+ enrichment ratio, were compared between the three colorgram levels using one-way ANOVA. Validating the colorgram level targeting, the product Hct was significantly different between the three colorgram levels. CD34+ CE2 and the CD34+ enrichment ratio trended higher with a lower Hct level. Lymphocyte CE1 trended higher with a higher Hct level. Our results are consistent with the hypothesis that CD34+ cells layer higher than lymphocytes in the collection interface, and that CD34+ cells may perhaps be more precisely targeted by collecting lighter in the collection interface. Our results pave the groundwork for further study with a more precise CD34+ CE1 calculation.

1 | Introduction

With cellular therapy collections, modifying technical collection parameters can improve target cell collection efficiency (CE). We previously showed that, with hematopoietic progenitor cell (HPC) collection in normal donors, adjusting the collect flow rate (CFR) based on the mononuclear cell (MNC) count rather than the WBC count improved MNC CE but not CD34+ CE; the only predictors of CD34+ CE were peripheral blood (PB) CD34+ count and product platelet count [1]. Other reported predictors of CD34+ CE in normal donors are PB Hct [2] and PB white cell count [3]. The association of product platelet count with CD34+ CE but not monocyte or lymphocyte CEs (which were associated with product granulocytes and product Hct, respectively) suggested that CD34+ cells may layer above monocytes and lymphocytes. Collection preference was not specifically examined in our previous study; however, in that the target colorgram level (level of redness) was not specified for the collections. Thus, the question of whether CD34+ cells inhabit a specific density within the MNC layer could not be investigated.

This study was therefore undertaken to determine, when using a CFR that controls for MNC count, whether targeting a specific colorgram level within the MNC layer might improve CD34+ CE without significantly increasing CEs of non-target cells. Improving CD34+ CE allows achievement of the target CD34+ goal with a lower blood volume processed, thereby improving patient/donor safety and operational efficiency. The level of redness is a surrogate for hematocrit (Hct) and signifies a certain depth within the MNC layer. An increase in CD34+ CE at

a particular colorgram level signifies that CD34+ cells may be most concentrated at that particular level. We also examined an additional novel surrogate to evaluate the level of the CD34+ cells, which we call the CD34+ enrichment ratio.

2 | Study Design and Methods

In 90 consecutive National Marrow Donor Program HPC collections performed in 2023 using the continuous MNC (CMNC) procedure (version 11.3) of the Spectra Optia, the MNC interface target, that is, the level of the MNC layer targeted for collection, was systematically rotated between three different colorgram levels; specifically, the three middle colorgram levels of the Spectra Optia collection preference tool (Terumo BCT, Part No. 777379057A) (Figure 1). Liters to process to reach the target CD34+ goal was calculated per our validated prediction algorithm [4]. The device's default packing factor (measure of centrifuge speed) of 4.5 was used in all collections. The CFR was adjusted per the following proprietary formula derived by Terumo: 0.002 * MNC count ($10^3/\mu L$) * inlet flow rate (mL/min), a modification that corrects for MNC count and thus reduces MNC CE variability [1]. All statistical analyses were conducted using R (version 4.4.2) within RStudio (version 2024.09.1 + 394.pro7). Relevant collection characteristics and CEs were compared between the three colorgram levels using one-way ANOVA for continuous variables (Table 1). Chi-square test with Fisher's exact correction was used for categorical variables. When an overall significant difference was detected, Tukey's post hoc test was used for multiple comparison adjustment. All statistical tests were assessed with statistical significance set at a p-value of <0.05. The CD34+ CE calculation was a CE2 value:

$$\mathrm{CD34^+CE2} = \frac{\#\,\mathrm{CD34^+cells\ in\ product}}{\left(\text{whole blood volume processed} * \left[\mathrm{PB\ CD34^+}\right]\right)}$$

whereas all other CEs are CE1 values:

$$\mathrm{CE1} = \frac{\#\,\text{cell type in product}}{\left(\text{whole blood volume processed} * \frac{\left[\text{PB pre} - \text{cell type}\right] + \left[\text{PB post} - \text{cell type}\right]}{2}\right)}$$

Product CD34+ enrichment in relation to the peripheral blood was calculated as follows:

$$\mathrm{CD34^+enrichment\ ratio} = \frac{\%\,\mathrm{CD34^+\ cells\ of\ product\ MNC\ count}}{\%\,\mathrm{CD34^+cells\ of\ peripheral\ blood\ MNC\ count}}$$

3 | Results

Patient demographics and donor characteristics reported to influence CD34+ CE [1–3] were not significantly different between colorgram groups (Table 1). There was a single adverse event observed, mild citrate toxicity relieved by pausing the procedure and infusing additional calcium gluconate, occurring at the end of one of 5 procedures in the series where over five total blood volumes were processed. PB CD34+ counts and CFR, which are known predictive factors for CEs [1, 4], did not significantly differ between the three colorgram levels (Table 2). Product volume (mean 460 ± 183 mL), inlet flow rate (mean 59 ± 11 mL/min), inlet: AC ratio (mean 13:1 ± 1.4:1) were also not significantly different between groups. For the CFR, post hoc Tukey's analysis did not reveal any significant pairwise differences between the three levels.

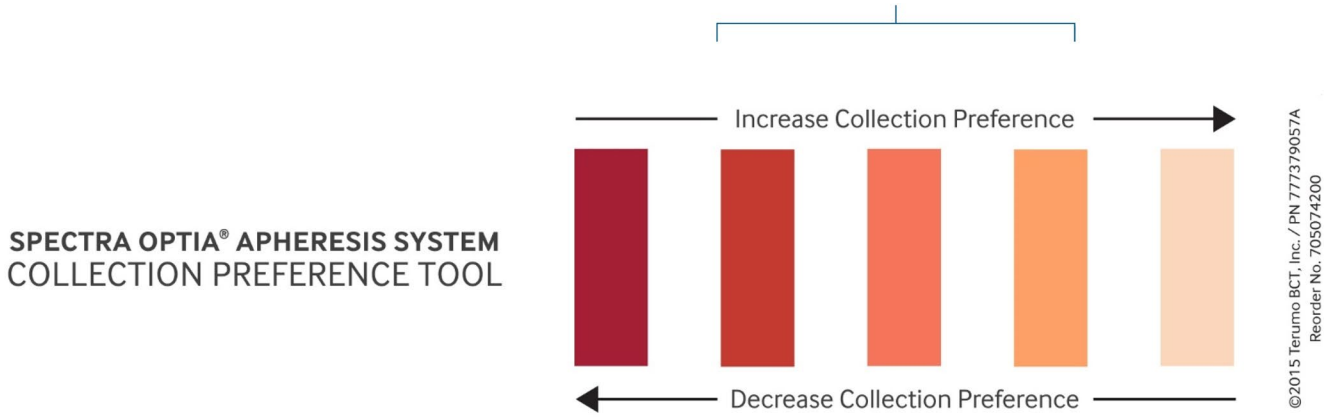

FIGURE 1 | Colorgram levels. Colorgram level is a surrogate for depth of collection within the MNC interface. HPC collections were rotated between the middle three colorgram bars.

TABLE 1 | Donor characteristics (mean ± SD).

Colorgram target	Age (year)	Male sex	TBV (mL)	Pre-Hct (%)	Pre-WBC ($\times 10^3/\mu L$)
Dark red ($n = 30$)	29 ± 7	57%	4968 ± 1033	42 ± 2	46 ± 15
Medium red ($n = 30$)	30 ± 7	67%	5128 ± 852	43 ± 1	46 ± 13
Light red ($n = 30$)	28 ± 7	67%	5102 ± 932	44 ± 1	48 ± 16
p-value	0.61	0.76	0.78	0.30	0.85

Note: Pre = 0–30 min pre-apheresis.
Abbreviations: SD, standard deviation; TBV, total blood volume.

TABLE 2 | Relevant collection characteristics (mean ± SD) and validation of colorgram targeting.

Colorgram target	PB MNC (10^3/μL)	PB CD34+ (/μL)	CFR (mL/min)	TBV processed	Product volume (mL)	Product Hct (%)	Product RBC (mL)	Product platelets ($\times 10^3$/μL)
Dark red ($n = 30$)	15.3 ± 5.0	91.3 ± 39.3	1.7 ± 0.6	2.6 ± 0.9	581 ± 147	6.5 ± 1.9	26 ± 11	1575 ± 663
Medium red ($n = 30$)	17.3 ± 6.0	98.7 ± 39.4	2.1 ± 0.8	2.4 ± 1.0	615 ± 207	5.8 ± 1.4	25 ± 9	1511 ± 537
Light red ($n = 30$)	18.3 ± 6.6	104 ± 57.4	2.1 ± 0.7	2.7 ± 1.3	694 ± 303	5.2 ± 1.3	26 ± 10	1450 ± 497
p-value	0.14	0.56	0.05	0.37	0.13	**0.008**	0.62	0.70

Note: Significant *p*-values are bolded.
Abbreviations: CFR, collect flow rate; Hct, hematocrit; PB, peripheral blood; RBC, red cells; SD, standard deviation; TBV, total blood volume.

TABLE 3 | Relevant product parameters (mean ± SD).

Colorgram target	Granulocyte %	Granulocyte CE1	Platelet CE1	Lymphocyte CE1	Monocyte CE1	CD34+ CE2	CD34 enrichment ratio
Dark ($n = 30$)	16.9 ± 8.7	0.05 ± 0.05	0.28 ± 0.09	0.51 ± 0.11	0.26 ± 0.08	0.58 ± 0.13	1.81 ± 0.78
Medium ($n = 30$)	16.1 ± 7.9	0.05 ± 0.03	0.29 ± 0.09	0.51 ± 0.13	0.28 ± 0.07	0.57 ± 0.10	1.76 ± 0.43
Light ($n = 30$)	14.5 ± 6.6	0.04 ± 0.02	0.30 ± 0.08	0.48 ± 0.12	0.28 ± 0.13	0.62 ± 0.12	2.04 ± 0.62
p-value	0.58	0.40	0.62	0.51	0.70	0.26	0.20

Abbreviations: CE1, collection efficiency 1; CE2, collection efficiency 2; SD, standard deviation.

Validating the colorgram level targeting, the product Hct was significantly different between the three colorgram levels, but not to the extent that disparity in red cell contamination was a significant concern. Despite differences in product Hct, product RBC volume did not differ significantly between groups, presumably due to variation in product volume.

There were no significant differences in the CE1s of non-target cells (granulocytes, platelets) between the three colorgram levels (Table 3); particularly notable safety-wise was no significant increase in platelet CE1 with a lighter collection interface. Although target cell CEs (CD34+ cells, monocytes, lymphocytes) and the CD34+ enrichment ratio at the three colorgram levels were not significantly different, their trends were consistent with our previous findings [1], with CD34+ CE2 and the CD34+ enrichment ratio trending higher with a lower Hct level and lymphocyte CE1 trending higher with a higher Hct level. These findings are consistent with CD34+ cells layering higher than lymphocytes in the collection interface.

Compared to a study [5] which used a fixed CFR of 2 mL/min, where the median CD34+ CE2 was 55% (range: 35.4%–111%), our median and average CD34+ CE2 were 59% (range: 26%–107%); the only two procedures with a CE2 < 40% (26% and 37%) were from the dark and middle colorgram levels, respectively. Compared to that study's median product volume of 540 mL (range: 211–865 mL), our median product volume was 415 mL (range: 198–914 mL). Therefore, although our product volume variability, as to be expected, was higher, our study had a higher median CD34+ CE2. Notably, 39 of 90 procedures (8 dark, 15 medium, 16 light) had a CFR > 2 mL/min, which, in addition to colorgram targeting, likely explains our higher CD34+ CE2 values.

4 | Discussion

This study systemically investigated whether, when using a CFR that controls for MNC count, targeting a light red colorgram level within the MNC layer improves CD34+ CE and without significantly increasing CEs of non-target cells. The CD34+ enrichment ratio was also calculated. Trends in this CE2-based study were consistent with our previous findings, which support proceeding with a larger and more precise (but costlier) study looking at CD34+ CE1 rather than CD34+ CE2—that is, where the post-apheresis CD34+ count is also measured and factored into the CE calculation. With such a study, the question as to the location inhabited by CD34+ cells within the MNC layer and whether their CE is improved by colorgram targeting may be investigated more thoroughly. This study also shows that tailoring the CFR to the MNC count rather than using a fixed CFR of 2 mL/min results in a lower median product volume and may improve CD34+ CE.

5 | Conclusions/Practitioner Points

1. The CD34+ enrichment ratio is a novel method to compare colorgram levels with hematopoietic progenitor cell collection.
2. Trends in this study using CD34+ CE2 and the CD34+ enrichment ratio were consistent with a light red colorgram level being targeted to enrich for CD34+ cells.
3. Tailoring the CFR to the MNC count rather than using a fixed CFR of 2 mL/min results in a lower median product volume and may improve CD34+ CE.

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Ethics Statement

IRB-exempt.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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