The Evaluation of Platelet Count as an Indicator of Iron Status in Voluntary Plateletpheresis Donors

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Abstract

Background: Iron deficiency is associated with lower mean cell volume (MCV), higher red cell distribution width (RDW), and higher platelet counts (PLT). Plateletpheresis units with high PLT are favored by donor centers due to increased split rates, however these donors may be at risk for iron deficiency. Establishing relationships between PLT and MCV, RDW, and ferritin could provide means to monitor donor iron status.

Methods: Phase 1 was a retrospective analysis of MCV, RDW, and PLT from plateletpheresis donors using hierarchical regression. Phase 2 was a prospective study of PLT and ferritin using log-linear regression. MCV, RDW, and ferritin were compared among platelet categories (singles, doubles, and triples).

Results: Retrospective results were obtained from 109 plateletpheresis donor records (1,309 donations). A significant relationship existed between PLT and MCV (p=0.001), indicating a PLT increase of $1x10^3/\mu L$ would result in an expected MCV decrease of 0.003fL. PLT and RDW also showed significance (p=0.01). PLT from the prospective analysis of 67 plateletpheresis donors demonstrated significant association with ferritin (p=0.013). PLT increase of $1x10^3/\mu L$ resulted in an expected ferritin decrease of 1.3%. Triple, double, and single units had ferritins of 33, 41, and 65 ng/dL, respectively. Ferritin levels between triple and single units approached significance (p=0.06).

Conclusion: Based on this study, for every increase in donor PLT of $100 \times 10^3/\mu L$, there would be an expected decrease in MCV and ferritin of 0.3fL and 73%, respectively. Higher donor PLT and plateletpheresis yields resulting in increased product split rates may be associated with a higher likelihood of donor iron deficiency.

Background

Iron deficiency has been well documented in voluntary whole blood (WB) donors by many research studies, however few studies have focused on plateletpheresis donors.[1-7] Plateletpheresis collections, using the TRIMA Accel Automated Blood Collection System (Terumo BCT, Lakewood, Colorado, USA), results in a minimum RBC loss of 30mL per procedure.[8] Although the risk of developing iron deficiency is less in plateletpheresis donors compared to WB donors due to this lower RBC loss, the effect is cumulative in regular plateletpheresis donors and may result in iron deficiency.

Iron deficiency, defined by a low ferritin level, is associated with lower mean cell volume (MCV), higher red cell distribution width (RDW), and higher platelet counts (PLT). Early stages of iron deficiency may not be detected by routine donor screening mechanisms.[4] Depletion of iron stores in blood donors occurs gradually until the most severe stage, iron deficiency anemia, is achieved. Decrease in iron stores to this extreme degree would result in low hemoglobin levels and exclude one from blood donation.

Although thrombocytosis (increased PLT) is a known sequalae of iron deficiency, its association with iron status in plateletpheresis donors and resultant platelet yields of the collected products has not been studied.[9] Collection of plateletpheresis units with high platelet yields are favored by donor centers due to increased product split rates. Studies have shown that frequent platelet donations of single or multiple products does not affect PLT, but the repeated RBC loss could create an iron deficient state and result in increased platelet production.[10,11]

Early detection of iron deficiency in plateletpheresis donors would be vital in maintaining donor iron stores and preventing future hemoglobin deferrals. This study evaluates the relationship between PLT and MCV, RDW and ferritin levels. Using platelet count as an indicator for iron status would be a non-invasive and cost-effective measure to monitor iron status in platelet donors.

Materials and Methods

Infrequent plateletpheresis donors from a hospital-based donor center were selected for the retrospective portion of this study if they had achieved 5 successful donations in the years 2012 and 2013. Platelet donors at this center can only donate platelets every 28 days, qualifying the program as infrequent per AABB standards.[12] This institutional review board (IRB)-approved analysis included collection of data from donor records, including MCV, RDW and PLT from each donation in 2012 and 2013. The selection of these parameters was based on the findings that MCV is a reliable indicator of iron status in healthy blood donors.[13] Donors were also categorized as either single, double or triple based on the split product rate of their donations.

A complete blood count (CBC) was performed at each donation to obtain the donor's platelet count (primary analyzer - ACTDiffII Hematology Analyzer, Beckman Coulter, Brea, CA, USA; backup analyzer – LH780 Hematology Analyzer, Beckman Coulter, Brea, CA, USA). RBC counts, RBC indices, and white blood cell parameters were extraneously obtained simultaneously as part of the routine CBC. These values were not routinely reviewed by the medical director.

The second part of this IRB-approved study was a short-term prospective analysis of platelet donors and their ferritin levels. After obtaining consent from the donor to participate in this

phase of the study, an additional sample tube was drawn during the platelet donation for ferritin testing. Ferritin levels were obtained spectrophotometrically using a Beckman Coulter AU680 (Brea, CA, USA). This portion of the study was performed in 2 separate waves – the first in the third quarter of 2014 and the second in the first quarter of 2015. Both sets of samples were analyzed exactly the same, and both data sets were evaluated using the same exclusion criteria. No subjects were repeated between the two time periods. Each donation was categorized as single, double, or triple based on the platelet yield of the final donated product.

Data obtained from the retrospective portion of this study were analyzed with a focus on relationships between both MCV and platelet count and RDW and platelet count. To assess the association between MCV and platelet count, a hierarchical regression model (including an additional error term to account for repeated observation of the same subjects) was fit using MCV as the response, platelet count as the predictor, and including controls for age and gender. A similar model was constructed using RDW as the response.

Data obtained from the prospective portion of this study were analyzed to evaluate the association between ferritin values and the corresponding platelet count. An overdispersed log-linear (Poisson) regression model was fit using ferritin as the response, platelet count as the predictor, and including controls for age and gender. The Poisson regression model was selected to account for the inherent skew in ferritin (skew=4.899), and the overdispersed option was used to account for the inflated mean-variance relationship (variance/mean = 232.397).

Mean PLT, MCV, and RDW from donors at each donation in the retrospective study as well PLT and ferritin values in the prospective study were analyzed based on the single/double/triple

product group designation using a 2-sample unequal variance t-test. Cohen's *d* and *r* values were used to verify significance. Donors from the prospective study were divided into two groups based on iron status and a 2-sample unequal variance t-test was used to analyze mean PLT. Significance was defined as $p<0.05$.

Results

Retrospective PLT, MCV, and RDW results were obtained from 109 plateletpheresis donor records representing 1,309 total donations at a 10,000 units/year blood donation facility. MCV and RDW values were separately analyzed in relation to the PLT to evaluate each parameter's effect. Mean values for all three parameters are shown for age and gender in Tables 1a and 1b, respectively. Age groups were chosen to represent the major age groups representative of the blood center's donor population.

According to the hierarchical regression model, a significant negative or inverse relationship between PLT and MCV was found ($t^*(1297) =-2.92$, $p=0.001$). The estimated coefficient value of -0.003 demonstrated an approximate nominal relationship between the two parameters where an increase of $1x10^3/\mu L$ in PLT would result in an expected decrease of 0.003 fL in MCV. Controls showed a significant association for age ($t^*(1297) =2.36$, $p=0.009$) but not for gender ($t^*(1297) =0.17$, $p=0.433$).

The hierarchical regression model for RDW showed significance for PLT ($t^*(1297) =-2.33$, $p=0.009$), with an estimated coefficient value of -0.002, meaning an increase of $1x10^3/uL$ in PLT would result in a decrease of 0.002% in RDW. Controls showed a significant association for age ($t^*(1297) =2.29$, $p=0.011$) but not for gender ($t^*(1297) =1.07$, $p=0.143$).

Longitudinal analysis of subsequent donations from each donor was performed. These longitudinal models for MCV and RDW were augmented to include a term evaluating a linear time effect. For the longitudinal model of MCV, the time effect was found to be -0.155 with an associated p value of $p < 0.001$. Accounting for the effects of platelet count, gender, and age, there was a significant decrease in expected MCV as times of observation increased. For each additional time of observation, the expected MCV would decrease by 0.155 (i.e. for each 10 donations, the MCV would be expected to decrease by 1.55fL). The effect of time was not found to be significant for the RDW model.

For the second phase of this study, 69 total platelet donors had an additional sample tube drawn for ferritin testing. Tables 2a and 2b show mean ferritin values and PLT among age groups and gender, respectively. Two donors were excluded from the study, both for exceeding the upper limit of the ferritin normal reference range (894 and 422 ng/mL). Elevated ferritin values are suggestive of possible disease processes or disorders unrelated to platelet donation. These donors were notified of their results and referred to their primary care physicians for follow-up.

Of the 67 donors included in the analysis, 35 (52%) had ferritin levels less than or equal to 26ng/dL, and therefore would be considered to have low ferritin or iron depletion. This was more common in the younger female donors in the age range of 18-35 years (80% of age group, 27% of females, 12% overall) and 36-50 years (83% of age group, 17% of females, 7% overall), however it was noted in both genders and all age ranges (see Table 2c). Mean PLT were higher in the low ferritin groups compared to the iron replete groups (p=0.008; Table 2d).

According to the log-linear regression model, PLT showed a significant association with ferritin (t*(65) =-2.55, p=0.013), with a coefficient of -0.013. Within the log-linear model, this means that an increase of $1x10^3/\mu L$ in platelet count was associated with an expected decrease in ferritin by a factor of exp(-0.013) = 0.987, or a decrease of about 1.3%. Following this model, an increase of $10x10^3/\mu L$ in platelet count would result in a decrease of about 12.2% in ferritin value, and an increase of $100x10^3/\mu L$ would result in an expected decrease of ferritin by 73%. Controls showed no evidence of a significant association for age (t*(65) =-1.13, p=0.264), and similarly for gender (t*(65) =-0.544, p=0.588).

Analyses of donor PLT, MCV, RDW, and ferritin levels by single/double/triple platelet products are presented in Tables 1c (retrospective study) and 2e (prospective study). In the retrospective study, PLT comparisons between single/double/triple platelets were all significant ($p \leq 0.0001$ for singles versus doubles and triples, 0.0003 for singles versus doubles). MCV was significant between singles and doubles and singles and triples ($p \leq 0.0001$ for both). RDW showed significance between double and triple platelets (p=0.04) and approached significance in the single versus double platelet analysis (p=0.05). Although mean MCV and RDW values from this data set showed very little variation, statistically significant p values were obtained. In order to verify the effect size of the t-test, Cohen's *d* and *r* were calculated (data not shown) and supports significance. Data from the prospective study demonstrated a decrease in mean ferritin levels as the platelet yields increased. Mean ferritin levels between single and triple platelet donations approached significance (p=0.06).

Discussion

Preventing iron deficiency in blood donors is a challenge, however the impact on plateletpheresis donors is minimized since a smaller volume of red cells are lost with each procedure. This focused study demonstrated that iron deficiency is a concern especially for younger, female plateletpheresis donors.

Both retrospective and prospective data analysis from this study indicated that PLT served as a surrogate indicator of iron status in voluntary plateletpheresis donors. The retrospective data analysis demonstrated a significant inverse relationship between PLT and MCV, where an increase in PLT correlated with a decrease in MCV. Controls accounting for age were found to be significant, but gender showed no discernible difference. As shown in Tables 1a and 1b, the mean values for all three parameters were relatively consistent for both age and gender. The one exception was a slight decrease in the mean PLT as age increases. This was also observed in the prospective data analysis and is displayed in table 2a.

In analyzing RDW, we expected to see an increase in platelet count to correlate with an increase in RDW. This conjecture was based on the observations of some researchers that RDW is a sensitive indicator of microcytic, hypochromic anemia.[14] However, a significant relationship in the opposite direction was observed. Similar to MCV, significance was seen with age but not gender. Since donors evaluated in this study were regular plateletpheresis donors having donated 5 or more times in the previous 2 years, it is possible that by the time the PLT began to increase in response to iron depletion or deficiency, the decrease in RBC size (reflected in the MCV measurement) had been present longer than 4 months and thus the RDW was not increased.

Analysis of ferritin values obtained during the prospective portion of this study revealed a significant inverse relationship where an increase in PLT was associated with a decrease in ferritin level. Although no statistical significance was specifically seen with either age or gender, Table 2a shows a decreasing trend in the mean PLT and increasing trend in ferritin as age increases. Additionally, Table 2b shows a lower mean ferritin level among women paired with a higher PLT when compared to male subjects in the study. Lower ferritin levels among women are expected, but the higher PLT further supports the relationship seen in this study.[15] Lower ferritin values were associated with higher PLT and product split rates (approached significance), however, larger studies are needed to confirm this observation.

The human body requires approximately 30 mg of iron per day to produce hemoglobin and other iron-containing proteins. Any "excess" iron is stored as ferritin.[16,17] Male and female whole blood donors lose approximately 240 and 220 mg of iron with each donation, respectively.[15] Although platelet donation results in less blood loss, procedures can be performed more often and a concurrent RBC may be collected if the donor is eligible.[6] There are several tests available to assess iron status, but ferritin is a relatively inexpensive, readily available test performed by most laboratories. World-wide, ferritin is the most common laboratory test used in the diagnosis of iron deficiency.[18]

External variables that can affect ferritin levels not controlled for in this study include dietary intake of iron, alcohol consumption, and a person's body mass index.[19] In addition, inflammation can cause falsely increased measurements of serum ferritin since ferritin acts as an acute phase reactant.[18] Subjects included in this study were healthy, volunteer blood donors. No donors

known to have or suspected to have hereditary hemochromatosis were included in this study. Two donors were excluded from the study and referred to primary care physicians due to elevated ferritin levels.

The blood center's current efforts to mitigate iron deficiency in platelet donors consist of monitoring the RBC loss after each donation using the Haemonetics El Dorado Donor software (Braintree, MA, USA). The RBC loss is calculated using information from the donation, such as whether a rinseback was performed and if a concurrent RBC product was donated. The absolute red cell dose of any concurrent RBC product is directly measured and entered into the donor's profile to yield a final value, not to exceed 1,695 mL annually.

Despite the monitoring of RBC losses which allows for intervention from the facility's quality coordinator and medical director if a donor is approaching the annual limit, platelet donors are still at risk of iron deficiency. The relationship observed in this study between MCV and ferritin levels and PLT may offer another approach for monitoring and preventing an iron deficient state in voluntary platelet donors.

Based on a study of ferritin measurement in WB donors by O'Meara and colleagues, levels below 10ng/mL showed a 93.5% specificity for iron deficiency anemia in men and 83.6% in women.[20] Using this definition of iron deficiency, 7 out of 67 donors (10%) in the prospective portion of this plateletpheresis study had ferritin levels indicative of iron deficiency. In the recent Hemoglobin and IRON Recovery Study (HEIRS), "low ferritin" and "iron replete" were defined as ferritin levels of ≤ 26 and >26 ng/dL, respectively.[21] Using this criteria for categorization, 35 of 67 (52%) of the

plateletpheresis donors had low ferritin or iron depletion. The mean PLT in the low ferritin cohort was 276 versus 246 overall (p=0.008), as displayed in table 2d.

Additional and larger studies are still needed to evaluate the complicated mechanism of iron deficiency and its relationship to thrombocytosis. There is no agreement yet among the blood bank community of the best way to monitor and mitigate the risk of iron deficiency in the donor population. In the meantime, blood centers need to be aware that higher donor PLT and plateletpheresis yields resulting in increased product split rates may be associated with a higher likelihood of donor iron deficiency.

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Table 1a: MCV, RDW, and PLT by Donor Age at Time of Study Enrollment (Retrospective Study)

Laboratory Parameters mean (range)	Age in years		
	18-35 n=16	36-50 n=12	>50 n=81
MCV fL	88 (77-104)	91 (75-100)	91 (73-106)
RDW %	13 (12-17)	14 (12-17)	14 (12-20)
PLT x 10^3/µL	269 (180-391)	262 (181-442)	242 (110-512)

MCV – mean corpuscular value, RDW – red cell distribution width,
PLT – platelet count

Table1b: MCV, RDW, and PLT by Donor Gender at Time of Study Enrollment (Retrospective Study)

Laboratory Parameters mean (range)	Gender	
	Female n=40	Male n=69
MCV fL	90 (73-106)	91 (75-104)
RDW %	14 (12-20)	14 (12-19)
PLT x 10^3/µL	243 (159-391)	249 (110-512)

MCV – mean corpuscular value, RDW – red cell distribution width,
PLT – platelet count

Table 1c: PLT, MCV, and RDW by Plateletpheresis Product Yield (Retrospective Study)

Donor Laboratory Parameters mean (range)	Product Yield		
	Single n=728	Double n=514	Triple n=67
PLT x 10^3/µL	234 (110-480)	254 (159-385)	297 (156-512)
MCV fL	91 (75-104)	89 (73-106)	89 (78-99)
RDW %	14 (12-20)	14 (12-20)	14 (12-16)

PLT – platelet count, MCV – mean corpuscular value, RDW – red cell distribution width
Comparison of donor PLT between single vs. double products $p < 0.0001$; double vs. triple $p = 0.0003$; single vs. triple $p < 0.0001$
Comparison of donor MCV between single vs. double products $p < 0.0001$; double vs. triple $p = 0.33$; single vs. triple $p = 0.0001$
Comparison of donor RDW between single vs. double products $p = 0.05$; double vs. triple $p = 0.04$; single vs. triple $p = 0.4$

Table 2a: Ferritin and PLT by Donor Age (Prospective Study)

Laboratory Parameters mean (range)	Age in years		
	18-35 n=17	36-50 n=13	>50 n=37
Ferritin ng/mL	34 (4-104)	53 (7-190)	51 (3-261)
PLT x 10^3/µL	273 (182-399)	264 (202-376)	256 (185-354)

PLT – platelet count

Table 2b: Ferritin and PLT by Donor Gender (Prospective Study)

Laboratory Parameters mean (range)	Gender	
	Female n=30	Male n=70
Ferritin ng/mL	35 (3-261)	57 (7-246)
PLT x 10^3/µL	284 (222-399)	243 (182-339)

PLT – platelet count

Table 2c: Ferritin Levels by Age and Gender

Number (%) of Donors (n=67)	Ferritin ng/dL	
	≤26	>26
Females		
18-35 yrs	8 (12)	2 (3)
36-50 yrs	5 (8)	1 (1)
>50 yrs	5 (8)	9 (14)
Males		
18-35 yrs	3 (4)	4 (6)
36-50 yrs	1 (1)	6 (9)
>50 yrs	13 (19)	10 (15)

Ferritin ≤26 represent low ferritin or iron depletion.
Ferritin >26 ng/dL represent iron replete status.

Table 2d: PLT by Gender and Ferritin Level

Gender and Ferritin Level ng/dL	Mean (range) PLT x 10^3/µL	p value
Females		
≤26	294(228-399)	0.15
>26	270(222-332)	
Males		
≤26	257(182-316)	0.04
>26	232(185-339)	
All Donors		
≤26	276(182-399)	0.008
>26	246(185-339)	

PLT – platelet count
Ferritin ≤26 represent low ferritin or iron depletion.
Ferritin >26 ng/dL represent iron replete status.

Table 2e: PLT and Ferritin Values by Plateletpheresis Product Yield (Prospective Study)

Donor Laboratory Parameters mean (range)	Product Yield		
	Single n=21	Double n=38	Triple n=8
PLT x 10^3/µL	229 (182-275)	271 (195-399)	301 (231-374)
Ferritin ng/mL	65 (7-261)	41 (3-247)	33 (7-62)

PLT – platelet count
Comparison of donor ferritin levels between single vs. double products p = 0.15; double vs. triple p=0.49; single vs. triple p=0.06.