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Donor platelet and leukocyte count as predictive factors of the quality of platelet concentrates obtained from whole blood by semiautomated fractionation

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ABSTRACT

Platelet concentrates (PCs) obtained from whole blood are produced by fractionation of the buffy coat (BC) or the platelet-rich plasma. Despite the improvements in the technologies used for the hemocomponent fractionation, the proportion of PCs that do not accomplish the quality requirements is high. This study aimed to determine whether the basal platelet and leukocyte counts are predictive factors of the quality of the PCs obtained from BC by semiautomated fractionation. Quality control registers of 196 PCs were analyzed. Gender- and age-dependence of the blood cell count and the characteristics of PCs were evaluated. Platelet yield and residual leukocytes in the PCs were correlated with the platelet and leukocyte counts and the age of the donors. Predictive efficacy was assessed, and an optimal cut-off was established. The proportions of PCs accepted and rejected by using or not the optimal cut-off were compared. 50.0% of the PCs accomplished all the quality control requirements. Female donors had a higher basal platelet count than males. A correlation was observed between basal platelets and platelet yield, but not between basal leukocytes and residual leukocytes. The basal platelet count predicted the quality of the PCs. A cut-off of 231,000 platelets/mm^3 was established, but it did not improve the proportion of accepted PCs. In conclusion, we found that the basal platelet count is correlated with the platelet yield. The basal leukocyte count is not correlated with the residual leukocytes. The established cut-off for the basal platelet count did not improve the proportion of accepted PCs.

1. Introduction

Platelet concentrates (PCs) are the third most transfused hemocomponent in Mexico, after the packed red blood cells (PRBCs) and the fresh frozen plasma (FFP); just in 2010, 505,417 PCs were transfused in our national territory [1]. PCs are composed of platelets, obtained from a blood donor, dispersed in about 50 mL of plasma mixed with anticoagulant [2]. Besides platelet apheresis, the primary methods used for PC obtention are the fractionation of platelet-rich plasma (PRP) and the fractionation of the buffy coat (BC) [3]. To obtain a PC from PRP implies that the whole blood needs to be centrifuged slowly, to precipitate the red blood cells and the leukocytes, leaving the platelets dispersed in the plasma, which is separated and then centrifuged again, to concentrate the platelets [3]. On the other hand, to obtain a PC from BC, fast centrifugation of the whole blood is needed, after which, the BC is obtained. Then, it is let to rest several hours and then is resuspended and recentrifuged slowly, to get the PC [4].

Despite the therapeutic advantages given by the apheresis-obtained PCs (a drastically diminished risk of alloimmunization), the production cost of these units is significantly higher than the production cost of PCs obtained from whole blood, in addition to a higher discomfort to the donor [5].

There is inconclusive evidence favoring the use of BC instead of PRP to produce PCs, although it has been reported that BC-obtained PCs have

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a higher quality [5]. Because the production from BC implies a long rest time for the blood before fractionation, platelets can reverse the aggregation process started during the phlebotomy, producing PCs with a higher platelet count compared to immediately fractioned blood units [6].

Despite the improvements in the technologies used for the hemocomponent fractionation, the proportion of PCs that do not accomplish the quality requirements is still high. A study performed in 2009 showed that 78.2% of the PCs obtained from PRP and 83.9% of the obtained from BC accomplish a quality requirement of more than 5.5×10^{10} platelets per concentrate [4]. For that reason, studying the causes of this low rate of quality acceptance is imperative to implement corrective actions impacting on the efficiency of PCs production. It is known that the basal platelet count of the donor is a predictive factor of the final platelet yield in PCs obtained by apheresis [7]; however, to our knowledge, the variables influencing the quality of the whole-blood-obtained PCs have not been studied comprehensively in Mexican population. This study aimed to determine whether the basal platelet and leukocyte count is a predictive factor of the quality of the PCs obtained from the BC.

2. Material and methods

This study was performed in the Blood Bank of the Department of Clinical Pathology of the University Hospital "Dr. José E. González, and it was reviewed and approved by the Ethics and Research Committee of our institution (Register number: PC20-00001). The quality control registers of single-donor PCs were evaluated, and data from January 2018 to January 2020 was obtained. To decide whether a PC accomplishes the quality requirements, the specifications of the Mexican Official Norm, NOM-253-SSA1-2012 were followed [8], considering acceptable a platelet yield of more than 6.0×10^{10} platelets per single-donor PC, a residual leukocyte count less than 5×10^7 cells, and an acceptable volume of more than 40 mL.

Both, the blood cell counts of the donors and the platelet and leukocyte counts in the PCs, were performed in a Cell-Dyn Emerald blood analyzer (Abbott). The whole blood units were obtained in GRIFOLS CPD – 450 quadruple bags, with a top & bottom system. The bags contained a solution of citrate-phosphate-dextrose as an anticoagulant and a solution of sodium chloride, adenine, glucose, and mannitol as PRBCs preserving solution. After phlebotomy, the whole blood units were allowed to rest for at last 60 minutes at room temperature. After the resting period, whole blood units were centrifuged in a Beckman Coulter J6-MI centrifuge, or a Presvac DP-2065R Plus centrifuge. Initial centrifugation (3,500 rpm, for 14 minutes at 22 °C) was performed to obtain the PRBCs, FFP, and BC. To obtain the PCs, the BCs were allowed to rest a period of 2 – 6 hours at room temperature, to favor the dissociation of platelet aggregates. Then, the BCs were centrifuged for 6 minutes at 1,000 rpm at 22 °C. All the units were fractionated in GRIFOLS Fractiomatic Plus 2 instruments by using the protocol 11 for BC obtention, and the protocol 15 for PC fractionation. PCs were kept in constant and slow swirling at 22 °C until their evaluation. No leukoreduction protocol other than centrifugation was performed during the production of PCs because leukocyte filters are used in our center during the transfusion of hemocomponents.

The basal platelet and leukocyte count of male and female donors, and the characteristics of their respective PCs were compared using a Student's *t*-test or a Mann-Whitney test, based on their distribution. The association of sex and the proportion of accepted and rejected PCs was assessed by using Fisher's exact test. A Spearman's rank correlation test was performed to correlate the platelet and leukocyte count in the PCs with the basal platelet and leukocyte counts in the donors, as well as with their age. An analysis of the areas under the receiving operating characteristic curve (AUROC) was done to evaluate the predictive efficacy of the association between the basal platelets and leukocytes with the platelet yield and residual leukocytes in the PC. An optimal cut-off was established in the donor platelet count to predict if the obtained PC will accomplish the quality requirement of platelet yield by using the method of maximization of the Youden's index. To compare the basal platelet and leukocyte count between accepted and rejected units, a Student's *t*-test was performed. Fisher's exact test was used to compare the proportions of PC accepted and rejected by using or not the optimal cut-off. Data were analyzed in the GraphPad Prism 7.04 software, considering significative a p-value < 0.05.

3. Results

A total of 196 single-donor PCs were analyzed, all of them obtained from the BC. Their general characteristics are described in Table 1. 151 PCs (77.04%) accomplished the quality requirement of platelet yield ($> 6.0 \times 10^{10}$ platelets per unit), and 138 PCs (70.41%) accomplished the quality requirement of residual leukocytes ($< 5 \times 10^7$ leukocytes per unit). Only one PC (0.51%) did not accomplish the quality requirement of a volume larger than 40 mL. Globally, only 98 PCs (50.00%) accomplished all the quality requirements evaluated. The basal platelet count was significantly higher in female donors than in the male. However, there was no significant difference between the platelet yield of PCs obtained from either female or male donors (Table 2). There were no differences between male and female donors regarding their basal leukocyte count and the residual leukocytes in their respective PCs (Table 2). Sex was found not associated with the rate of rejection, neither because of the platelet yield nor the residual leukocytes (Table 2).

3.1. Correlation between basal platelet, leukocyte counts, and age of the donor and final yields in the PC

Spearman's rank correlation coefficient of 0.3840 [95% confidence interval (95% CI): 0.2538 – 0.5005; $p < 0.0001$] was observed after correlating the basal platelet count of the donor with the PC platelet yield. A significative correlation between the basal leukocyte count of the donor and the residual leukocytes in the PC was not observed ($\rho = 0.0345$; 95% CI: -0.1103 – 0.1778; $p = 0.6314$). No significant correlation was observed between neither the age and the platelet yield ($\rho = -0.0279$; 95% CI: -0.1715 – 0.1168; $p = 0.6977$) nor the residual leukocytes in the PC ($\rho = 0.0664$; 95% CI: -0.2087 – 0.07854; $p = 0.3547$).

Table 1
General characteristics of the assessed PCs

Parameter	Value
Platelet yield (IQR) – platelets/PC*	7.86×10^{10} (6.09×10^{10} – 9.80×10^{10})
Platelet count of the PCs – platelets/mm^3	$1.46 \times 10^6 \pm 0.46 \times 10^6$
Accepted (%)	151 (77.04)
Rejected (%)	45 (22.96)
Basal platelet count of the donor – platelets/mm^3	260,755 ± 52,610
Residual leukocytes in the PCs (IQR) – leukocytes/PC*	3.51×10^7 (3.16×10^7 – 5.18×10^7)
Leukocyte count of the PCs (IQR) – leukocytes/mm^3	600 (600 – 900)
Accepted (%)	138 (70.41)
Rejected (%)	58 (29.59)
Basal leukocyte count of the donor – leukocytes/mm^3	7,436 ± 1,452
Volume (IQR) – mL*	56.5 (53.8 – 58.5)
Accepted (%)	195 (99.49%)
Rejected (%)	1 (0.51%)
PCs fully approved	98 (50.00)

* Expressed as median (interquartile range). All the other values are expressed as mean ± standard deviation. PC: Platelet concentrate; IQR: Interquartile Range.

Table 2
Characteristics of the assessed PCs subdivided by sex

Parameter	Female (n = 36)	Male (n = 160)	*p*-value
Platelet yield (IQR) – platelets/PC*	9.15×10^{10} (5.60×10^{10} – 1.30×10^{11})	7.82×10^{10} (6.13×10^{10} – 9.62×10^{10})	0.3705
Platelet count of the PCs (IQR) – platelets/mm^3*	1.61×10^6 (1.06×10^6 – 1.94×10^6)	1.41×10^6 (1.15×10^6 – 1.72×10^6)	0.4062
Accepted (%)**	26 (72.2)	125 (78.1)	0.5109
Rejected (%)**	10 (27.8)	35 (21.9)	
Basal platelet count of the donor – platelets/mm^3	289,444 ± 66,780	254,300 ± 46,739	**0.0002**
Residual leukocytes in the PCs (IQR) – leukocytes/PC*	3.52×10^7 (3.23×10^7 – 5.32×10^7)	3.51×10^7 (2.92×10^7 – 5.18×10^7)	0.5185
Leukocyte count of the PCs (IQR) – leukocytes/mm^3*	600 (600 – 900)	600 (600 – 900)	0.5703
Accepted (%)**	24 (66.7)	12 (33.3)	0.6864
Rejected (%)**	114 (71.2)	45 (28.8)	
Basal leukocyte count of the donor (IQR) – leukocytes/mm^3*	7,800 (6,975 – 8,475)	7,200 (6,225 – 8,375)	0.0571
Volume (IQR) – mL*	57.0 (53.8 – 59.3)	56.5 (53.8 – 58.5)	0.3962
Accepted (%)**	36 (100.0)	0 (0.0)	>0.9999
Rejected (%)**	159 (99.4)	1 (0.6)	
PCs fully approved (%)**	15 (41.7)	83 (51.9)	0.3565

* Expressed as median (interquartile range). All the other values are expressed as mean ± standard deviation. ** Fisher's exact test. PC: Platelet concentrate; IQR: Interquartile Range.

3.2. Evaluation of the basal platelet and leukocyte counts of the donor as quality predictors

The use of the basal platelet count as a quality predictor showed a significative AUROC of 0.6040, while the basal leukocyte count did not show a significative AUROC, having a value of 0.5222 (Fig. 1). When comparing the basal platelet count of the donors from whom PC with acceptable and non-acceptable platelet yields were obtained, a significative difference was found, with a *p*-value of 0.0179 (Fig. 2). On the other hand, when comparing the basal leukocyte count of donors from whom PC with acceptable and non-acceptable residual leukocytes were obtained, there was no significant difference ($p = 0.7637$, Fig. 2).

3.3. Performance of the basal platelet count of the donor as a quality predictor

An optimal cut-off of 231,000 platelets/mm^3 was established to predict whether a PC will accomplish or not the quality requirement of the platelet yield. The use of the basal platelet count showed a sensitivity of 78.81% (95% CI: 71.62 – 84.57%), a specificity of 46.67% (95% CI: 32.94 – 60.92%), a positive predictive value of 83.22% (95% CI: 76.24 – 88.45%) and a negative predictive value of 39.62% (95% CI: 27.59 – 53.06%) for the prediction of an acceptable platelet yield. When comparing the proportion of PCs accepted and rejected using the optimal cut-off (119 accepted, 24 rejected) versus the proportion without using the cut-off (151 accepted, 45 rejected), there was no significant difference ($p = 0.1744$).

4. Discussion

Fractionation of the BC is a widely spread technique to obtain PCs around the world, mainly because of its advantages over the PRP method [9]. It is the most used methodology to produce PCs in Europe, alongside platelet apheresis. A report of 2005 showed that in countries like Germany, 44.1% of all the PCs were obtained by BC fractioning, 48.6% were obtained by apheresis, and only 7.2% were obtained from PRP [10]. Contrarily, in the United States, the PRP fractionation is still the principal methodology to obtain PCs [11]. Several reports indicate that the production of PCs from BC has substantial advantages compared with their production from PRP [6,12]. It has been reported that fractionation of the BC produces PCs with a quality comparable to apheresis-obtained PCs [4]. In Mexico, most blood banks produce single-donor PCs from BC instead of pooled PCs, following the requirements of the NOM-253-SSA1-2012 [8]. Many countries rely on pooled PCs for platelet transfusions, however, the characteristics of pooled PCs depend on the quality of the PCs used in the pool, hence the results of this study are still appliable to the blood banks using pooled PCs.

Nowadays, semiautomated fractioning by top & bottom technologies allows to accelerate the process of production of PRBCs, FFPs, and BCs, from which PCs, are obtained, however, those methodologies imply random errors by the instrument and by the operator, which, in addition to the biologic variability of the donors, affect the quality control parameters. For that reason, the search for strategies to improve the quality of PCs is critical. In Mexico, the NOM-253-SSA1-2012 does not give details about the characteristics that a donor must have to the PCs

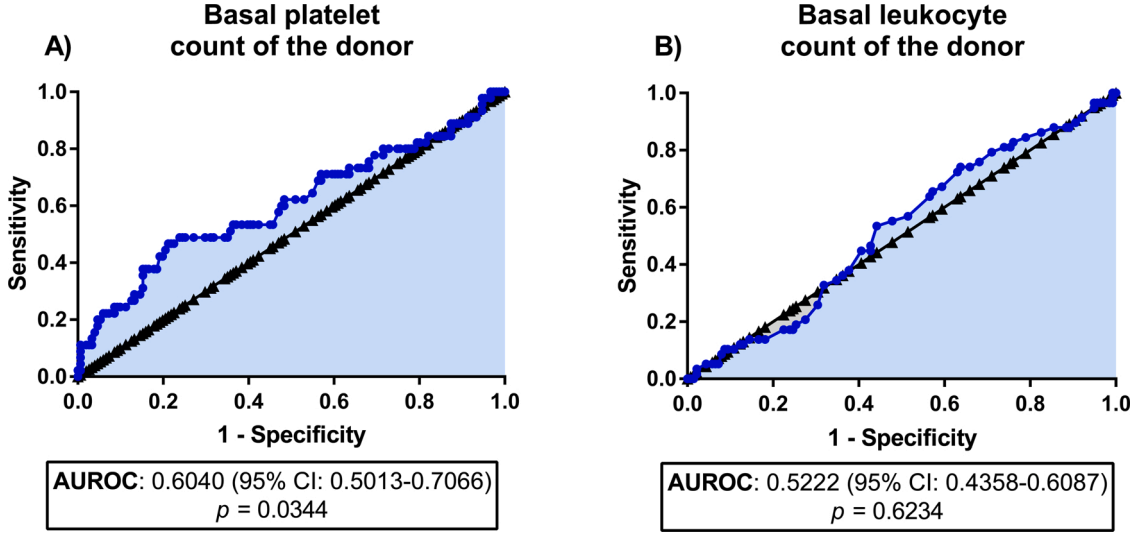

Fig. 1. Analysis of the AUROC for the evaluation of the basal blood cell counts of the donor as quality predictors of the platelet PCs. A) Efficacy of the basal platelet count to predict the platelet yield in the PCs. B) Efficacy of the basal leukocyte count to predict the residual leukocytes in the PCs.

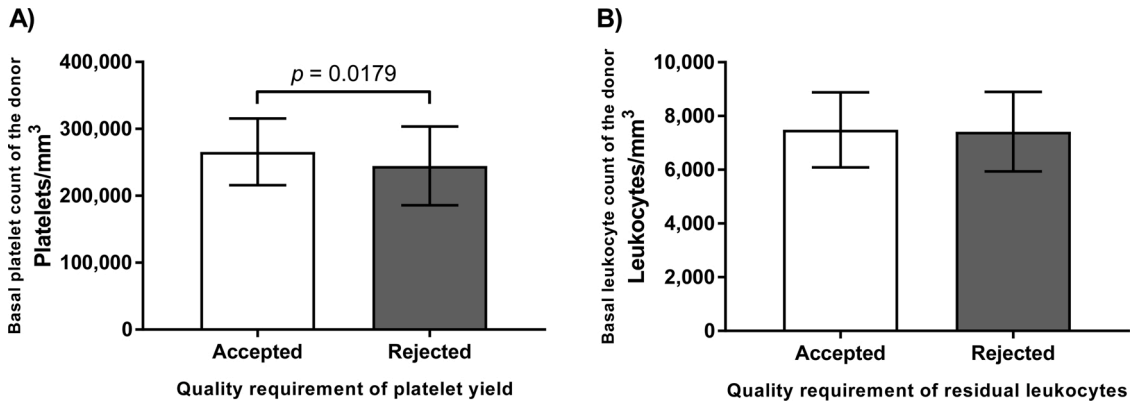

Fig. 2. Comparison of the basal platelet (A) and leukocyte (B) counts between PC with acceptable and non-acceptable platelet yields or residual leukocytes. Values are expressed as mean ± standard deviation.

obtained from the whole blood to accomplish the quality requirements [8]. Because of that, identifying factors of the donor associated with the quality of the PC is crucial to select the donors from which PCs will be fractioned, avoiding the production of low therapeutic quality PCs. In this project, it was observed that 77% of the PCs accomplished the minimum platelet yield, while only 70% accomplished the requirement of residual leukocytes. These results were similar to studies in which it has been observed that PCs obtained from BC have acceptable platelet yields in 70 – 90% of the assessed units [4,13]. On the other hand, it has been reported that more than 90% of the PCs have acceptable residual leukocytes [13], which differs from the results obtained in this study.

Our results showed that female donors have a significantly higher platelet count than males. This observation agrees with data previously reported in the literature, showing that platelet counts are gender-dependent [14]. It was observed that the basal platelet count of the donor is correlated with the platelet yield of the PC, but not the donor leukocyte count. It was also observed that the basal platelet count has a moderate, but significative efficacy to predict the final platelet yield in the PC, although the residual leukocytes cannot be predicted by knowing the basal leukocyte count. It can be inferred that the contamination of PCs with leukocytes occurs randomly, either by random errors of the fractionating instrument or by operator errors. The correlation between the basal platelet count and the platelet yield in the PCs was not strong enough to reflect the differences in the platelet count between female and male donors. Hence, we did not observe a difference, but a trend, in the platelet yield of PCs obtained from either female or male donors. The predictive efficacy of the basal platelet count has been observed in studies in which the quality control of PCs obtained by apheresis has been assessed [7], showing results similar to this study.

The use of a cut-off of 231,000 platelets/mm^3 to decide to fractionate or not a blood unit implies that around 83% of the units obtained from a blood donor with an acceptable platelet count will approve the quality control. When comparing the proportion of accepted and rejected PCs if this cut-off were used with the global proportion observed in this study, a statistically significant benefit was not observed. The cause of this could be random errors during the fractionation process. The use of fully automated fractioning instruments would decrease this variability, allowing to use the basal platelet count as a criterium to decide if the PC will be fractioned or not.

Because the official Mexican regulations regarding donor selection do not consider mandatory to assess the parameters of the complete blood count other than hemoglobin and hematocrit for whole blood donation, not every center has implemented the use of complete blood cell counts for the donor selection. The use of the complete blood cell count in our center has proven us useful to be an additional factor to consider for taking decisions regarding the quality control of hemocomponents.

5. Conclusions

The basal platelet count of a donor is correlated with the final platelet yield in the PC, but the basal leukocyte count is not correlated with the residual leukocytes. The optimal cut-off of the basal platelet count of a donor is 231,000 platelets/mm^3; however, it does not produce a significative benefit on the proportion of accepted and rejected PCs obtained by BC semiautomated fractioning.

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CRediT authorship contribution statement

Eduardo Cienfuegos-Pecina: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Erika Rubí Leal-Nava:** Conceptualization, Methodology, Resources, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Luz Elena Avilés-Rodríguez:** Conceptualization, Investigation, Resources, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition. **Jorge Martín Llaca-Díaz:** Conceptualization, Investigation, Resources, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition. **Fernando Pérez-Chávez:** Resources, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition. **Rogelio Cázares-Tamez:** Resources, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition. **Erik Alejandro Díaz-Chuc:** Conceptualization, Resources, Investigation, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

None.

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