ORIGINAL ARTICLE

Concordance between platelet counts by impedance and optical technique during microcytic anemia: Towards a threshold value of the mean corpuscular volume

Etude de la concordance entre la numération des plaquettes par impédance et par technique optique au cours des anémies microcytaires: Vers une détermination d'une valeur seuil du volume globulaire moyen

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Abstract

Introduction: Platelet count is crucial for clinical decision. In cases of microcytosis, platelet count based on impedance technique (PLT-I) may overestimate platelet count.
Aim: To compare PLT-I with platelet count using the optical technique (PLT-O) and establish a Mean Corpuscular Volume (MCV) threshold for considering PLT-O.
Methods: A prospective analytical study conducted over two months involved blood samples collected in standard K2 EDTA tubes for complete blood count analysis, revealing microcytosis (MCV<80 fL). PLT-O analysis in channel-Ret mode was performed using the Sysmex-XN1000 (Sysmex Corporation, Kobe, Japan).Percentage of fragmented red cells(FRC%) and percentage of microcytic red cells(Micro-R%) were recorded. Blood smears stained with May-Grünwald-Giemsa were examined for potential interfering particles.
Results: A strong correlation was observed between the two techniques for all platelet values as well as for PLT <150 x 109/L (correlation coefficient r = 0.971, 95% CI: [0.956-0.982]; P<10-3 and r = 0.90, 95% CI: [0.79-0.95]; P< 10-3). The Bland-Altman plot revealed a bias of 16.53 x 109/L between the two methods, with agreement limits between -55.8 and 88.8 x 109/L. A threshold MCV value indicating the use of the optical method, with a cut-off at 72.9fL, demonstrated promising performance consistent with litterature findings. However, less favorable performance was observed with Micro-R%.
Conclusion: Impedance could be employed in routine practice. However, for MCV<72.9 fL or in the presence of schizocytes, the hemogram validation procedure may incorporate the use of PLT-O.

Key words: platelets, impedance, optic, microcytosis, mean corpuscular volume, anemia

Résumé

Introduction: La numération plaquettaire est un examen très utile pour la prise de décision en clinique. En présence de microcytose, la technique d'impédance (PLT-I) peut surestimer la numération plaquettaire.
Objectifs: Etudier la concordance entre les PLT-I et la numération par technique optique (PLT-O) et déterminer un VGM seuil indiquant le recours aux PLT-O.
Méthodes: Etude prospective, analytique sur 2 mois ayant concerné des hémogrammes réalisés sur l'analyseur Sysmex-XN1000®, et dont le résultat montre un VGM<80fL. Les PLT-O ont été réalisés via le canal-Ret. Les résultats du pourcentage de globules rouges fragmentés (FRC%) ainsi que le pourcentage des globules rouges microcytaires (Micro-R%) ont été recueillis. Des frottis colorés au MGG ont été examinés afin de détecter la présence d'éventuelles particules interférentes.
Résultats: Unebonne corrélation a été observée entre les deux techniques pour tous les chiffres plaquettaires ainsi que pour les PLT<150 109/L (respectivement, r =0,971 , l'IC 95% : [0.956- 0.982] ; P <10-3 et r =0,90, IC 95%: [0.79- 0.95] ; P <10-3). Le graphique de Bland et Altman a révélé un biais de 16,53 109/L entre les deux méthodes avec des limites d'agrément entre -55,8 et 88,8 109/L.Un seuil du VGM à 72,9 fL indiquant le recours à la méthode optique, a montré des performances encourageantes rejoignant celles de la littérature.Le Micro-R% a montré des performances moindres.
Conclusion: L'impédance pourrait être utilisée en routine. Cependant, pour les VGM<72.9fL ou en présence de schizocytes, la procédure de validation de l'hémogramme pourrait integrer le recours aux PLT-O.

Mots clés:Plaquettes, Impédance, Optique,microcytose, volume globulaire moyen,Anémie

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Introduction

Platelet count is a standard procedure in hematology analysis across various clinical scenarios. Traditional manual methods, specifically phase contrast microscopy, have been replaced by automated techniques, including impedance or optical analyzers, known for their faster and more accurate results (1).

Generally, there are three technological principles (impedance, optical, and immunologic) that underlie the calculation of platelet (PLT) count. Impedance involves the identification of platelets as particles generating a recorded electrical pulse. This technique is widely employed in hematology analyzers. However, it is important to note that interference from particles like schizocytes, microcytes, spherocytes, and cellular debris, particularly when their sizes closely reache those of platelets, may lead to an overestimation of platelet counts (1–3).

Optical systems are less susceptible to interference since they use at least two dimensions (cell size and internal complexity) (4). However, the systematic use of this technique should be more codified as this measurement method requires an additional reagent whose cost should be studied.

Morerecently, flow cytometry techniquesusing labeled monoclonal antibodies havebeenproposed as the reference technique (5). The widespread adoption of flow cytometry is limited by its high cost. Consequently, platelet counts using impedance and optical techniques are more commonly employed in routine practice (6).

In the medical practice, microcytic anemia is frequently encountered. The existence of microcytes can lead to an overestimation of platelet counts, potentially concealing an underlying thrombocytopenia that may be associated (7).

This study aims to compare platelet counts obtained through impedance (PLT-I) and optical methods (PLT-O) in patients with microcytic anemia. Aditionally, we aimed to identify the critical mean corpuscular volume (MCV) value at which the utilization of optical methods becomes pertinent.

Methods

The research was conducted over two months, at the Haematology department of our institution. Blood samples anticoagulated with K2 EDTA were collected from specimens received from various medical and surgical departments inpatients, as well as outpatients.

We included blood counts from patients over 12 years of age whose results show microcytosis (MCV<80 fL) or microcytic anemia (hemoglobin levels <13g/dL in men and <12g/dL in women).

We excluded blood samples with insufficient quantity, which did not allow for a second analysis in the Ret channel. Non-compliant samples, such as coagulated, hemolyzed, icteric, and lipid samples, were also excluded.

For each sample, we performed a blood count on the Sysmex XN-1000® analyzer based on impedance and optical techniques. Colored blood smear with May Grunwald Giemsa were prepared to assess platelets morphology and detect interferent particles likely to disturb platelet counting such as schizocytes and microcytes.

Statistical analysis was carried out using Microsoft Excel and SPSS statistics version 23.0.

The Shapiro test was used to determine normal distribution of variables. Paired Student t test was used. Otherwise, Mann-Whitney U was realised. Correlation between PLT-I and PLT-O was investigated by Spearman coefficient r. The Bland Altamn plots were generated to evaluate concordance of detecting platelets by the two techniques. Receiver operating characteristic (ROC) curves of MCV and percentage of microcytic red cells (Micro-R%) and their performances were determined were generated by Medcalc. The area under the curve (AUC) were respectively determined. The optimal Cut-offs of MCV to predict overestimation of PLT-I was calculated.A P- value of < 0.05 was considered statistically significant.

The study protocol was in accordance with the Declaration of Helsinki.

Results

Our study involved 249 whole blood samples collected on K2 EDTA from inpatients from various medical and surgical wards, as well as from outpatients.

The mean age of patients included in the study was 49.39 ±17.86 years, ranging from 12 to 82 years. More than a third of patients were between 40 and 45 years of age.

This study included 160 women (64.25%) and 89 men (35.75%) with a gender ratio of 0.55.

Samples were categorized based on their MCV into three groups : 7.23% (n=18) had an MCV between 50 and 60 fL, 30.52% (n=76) had an MCV between 60 and 70 fL and 62.25% (n=155) had an MCV between 70 and 80 fL.

The average value of PLT-I was significantly higher than PLT-O (302.26±152.8*109/L and 285.68 ±154.27*109/L respectively with; P-value < 0.05).

A good positive correlation was noted between the impedance technique and the optical technique, with a correlation coefficient (r) of 0.971 and a 95% confidence interval of [0.956-0.982], with a statistically significant P-value of less than 10-3 (Figure 1A).

In the case of thrombocytopenia, the study showed a good correlation between the impedance technique and the optical technique (n= 27) with r =0.90, 95% confidence interval: [0.79- 0.95] with P <10-3 (Figure 1 B).

The Bland and Altman plot illustrated a bias of 16.53 between the two methods, with agreement limits ranging from -55.8 to 88.8. Figure 2 represents these findings.

Moreover, the dispersion of values is greater as the platelet count rises.

In addition, five cases of PLT-I overestimation exceeding the upper approval limit were recorded.

No underestimation by the impedance technique is detected below the lower approval limit.

It's worth noting that in the assessment of agreement between the two techniques for measuring platelets

in cases of thrombocytopenia, no significant bias was observed (bias=0.51). The agreement limits were found to be between -9.04 and 7.08. These results lead to the conclusion that there was a satisfactory agreement for platelet levels below 150*109/L.

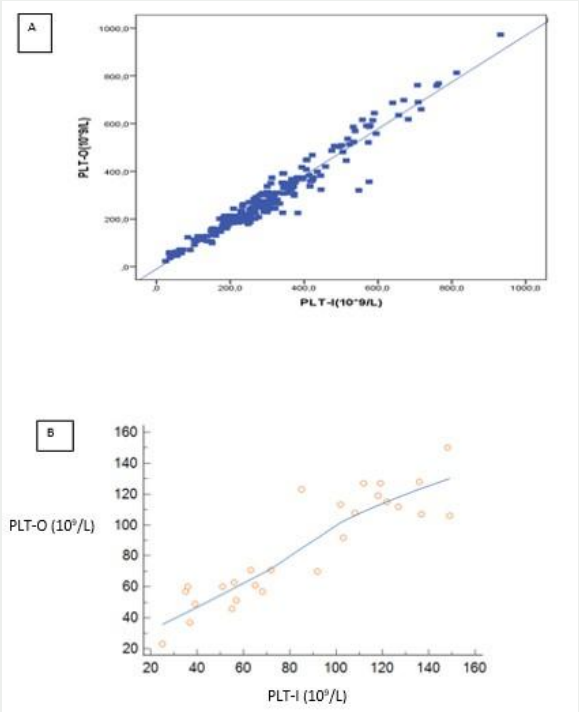

Figure 1. Correlation between platelet counts using PLT-O and PLT-I methods
A : in all patients ; **B** : in thrombocytopenic patients

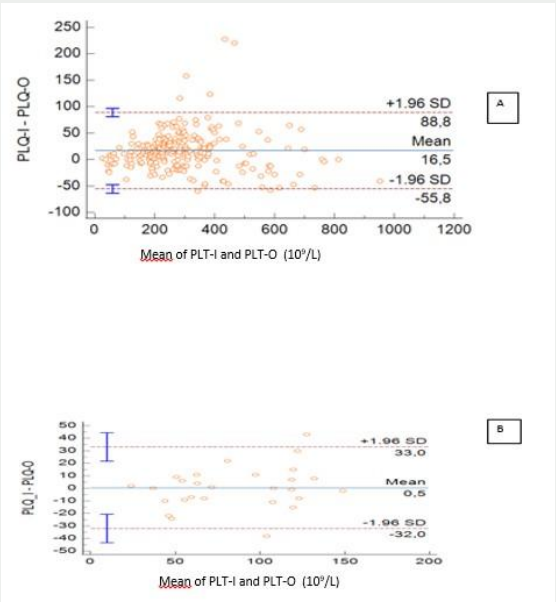

Figure 2. Bland-Altmann analysis comparing the impedance and the optical method
A: in all patients ; **B**: in thrombocytopenic patients

We wanted to study the concordance between PLT-I and PLT-O in the three levels of MCV values. The different graphical representations using Bland Altman's method are shown in figure 3.

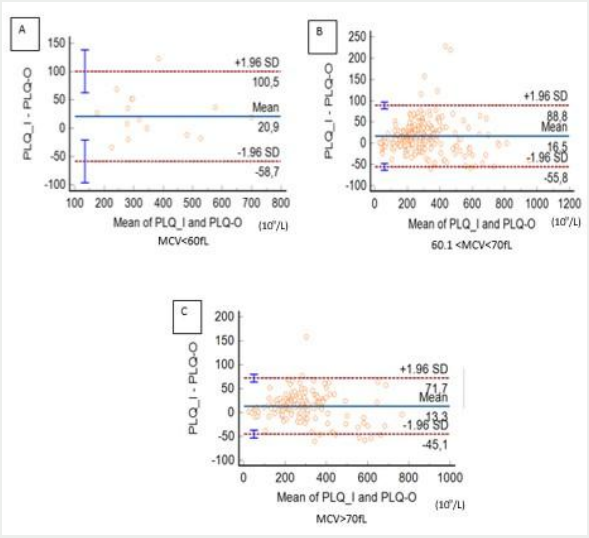

Figure 3. Bland Altma analysis: agreement between platelet counts and MCV for samples with A, MCV<60 fL ; B, for MCV between 60.1fL and 70 fL and C, for MCV over 70 fL

Upon observation of the graphs, a significant bias was evident between the two platelet measurement techniques, revealing an overestimation of 20.9*109/L by the impedance technique when the MCV is ≤60fL. The confidence intervals for the upper and lower agreement limits were considerably wide.
Conversely, for MCV values>60fL, the mean difference between the two techniques was lower, and the dispersion of values was reduced, indicating a more consistent and reliable agreement in this range.
In a second phase of the study, we wanted to determine a threshold value for MCV and the Micro-R% parameter, indicating that the optical platelet count could be performed as part of the biologist's validation procedure. He two ROC curves for MCV and Micro-R% respectively are shown in figures 4 (A) and 5 (B).
This shows that MCV can be used as a factor pointing towards an overestimation of platelet count by the impedance technique (i.e.ΔPLT-I-PLT-O> 0). (AUC:0.632; 95%CI: [0.568- 0.692]).

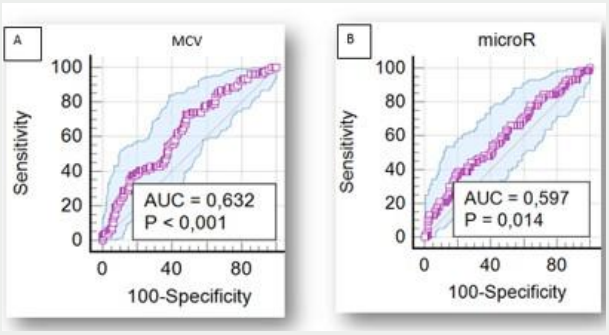

Figure 4. ROC curve of A, MCV and B, micro-R

The overall performance of the Micro-R% parameter in pointing to interference by the impedance technique appears more modest (AUC: 0.597; 95% CI: [0.533-0.658]).
The search for the optimum value for MCV and Micro-R% to indicate recourse to the optical technique (due to interference with the impedance technique) was carried

out via calculation of the Youden index.
This showed that when MCV≤ 72.9fL or Micro-R%>37.3%, the use of optical platelet measurement would compensate for the overestimation observed with the impedance technique. Table 1 summarizes the performance of the proposed cut-offs for MCV and Micro-R%.
This MCV cut-off gave good sensitivity, but low specificity. In contrast, the cut-off proposed for Micro-R% was associated with good specificity without achieving satisfactory sensitivity.

Table 1. Performance of proposed cut-offs for MCV and Micro-R% parameter

Parameters	Youden Index	proposed Cut-off	Sensitivity (%)	Specificity (%)
MCV*	0.24	≤ 72.9fL	72.73	51.74
Micro-R%**	0.18	>37.3%	38.96	79.65

Discussion

The platelet count is a clinically important parameter of the automated complete blood count. The impedance technique, employed for platelet counting, is extensively utilized due to its availability on all cellular hematology analyzers. Nonetheless, impedance counting has limitations as cell size analysis is unable to differentiate platelets from similarly sized particles like microcytes, spherocytes, or schizocytes. (8).
In this context, microcytic anemia can lead to an increased platelet count. This raises the question of the benefits of using optical techniques through the reticulocyte channel to count platelets (9).
This study aimed to compare platelet counts derived from both impedance and optical techniques among individuals with microcytic anemia. Additionally, the goal is to pinpoint the crucial mean corpuscular volume (MCV) threshold at which the adoption of optical methods becomes relevant.
This study indicates that the impedance method produced a higher platelet count when compared to the optical method. These results align with the observations made by Pinkowski et al., who reported that the optical method provides a more reliable platelet count in cases of microcytic anemia compared to the impedance method (10).
A good positive correlation was observed between the two techniques (figure 1), with a correlation coefficient (r) of 0.971 and a 95% confidence interval of [0.956-0.982], with p below 10-3. For cases of thrombocytopenia in our study (platelets below 150*109/L) the correlation coefficient between the two methods was slightly lower than the overall population, measuring at r=0.90. It's important to note that the sample size of thrombocytopenic patients is small (n=27), with only 5 patients exhibiting more severe thrombocytopenia (<50*109/L).
Hummel et al (3) demonstrated in a study involving 168 thrombocytopenic patients (PLT<35*109/L) that the impedance method wasn't able to determine platelet counts for levels below 10*109/L . However, another study revealed a higher correlation coefficient between PLT-I and PLT-O (r=0.82) for samples exhibiting severe thrombocytopenia (platelets less than 50*109/L). It is worth to mention, that this particular study excluded samples with interfering particles or microcytes (11).
In fact, severe thrombocytopenia decreases the statistical accuracy of platelet counting methods, especially by impedance, which can lead to statistical errors of 7-14%. On the other hand, the optical method can be adapted in this case by automatically increasing the count time on a richer cell suspension, which minimizes its statistical errors (1).
According to Bland and Altman's graph, impedance increased the optical technique's count by 16.5*109/L (figure 2). This finding has been made by other authors, but the bias varied considerably.
Indeed, a comparative study of 3 methods (impedance, optical and immunological) found a consistent overestimation of platelets by the impedance method compared with the immunological method (CD61), with a PLT-I:CD61 equal ratio of 1.25 on average, whereas the PLT-O:CD61 ratio was of the order of 0.87 (12).
A study by Pinkowski R et al found that impedance was on average 9.7% higher than the optical method (10) .
While a lower mean difference value of around 4.5*109/L was found in a French study. It should be mentioned that authors focused on thrombocytopenic subjects without prejudging the presence of associated microcytosis (1). The large difference between the values obtained by this study and our own emphasized the effect of microcytosis, which seems to be responsible for the significant increase in platelet count by the impedance technique.
Thus, it appears that there is a concordance between impedance and optical counts, with a variable increase with the impedance technique, which tends to be accentuated in the presence of microcytosis and for low platelet counts.
In our study, Bland and Altman's MCV graph showed an inversely proportional relationship between MCV and platelet count by impedance. A fairly low MCV indicates the existence of a very large number of microcytes likely to major platelet overestimation by impedance. (Figure 3). Our results corroborate those reported in the literature.
Indeed, the study by Tantanate C et al, evaluating the impedance against a reference immunological technique, found 125 cases of inaccurate results of the PLT-I in 249 thalassemic subjects with mean MCVs of 73.2 fL, 72.5 fL and 69.5 fL respectively (13).
However, in a few samples, platelet counts by the optical method were higher than those reported by the impedance method. In these cases, the interfering particles are rather giant platelets underestimated by the impedance method. Other studies suggest that leukocyte fragments may be responsible for the disruption of the optical technique (14,15).
We also investigated the profile of Micro-R% in microcytic anemia and microcytosis and its relationship with platelets. We were able to demonstrate that Micro-R% varied significantly as a function of MCV, which in turn can impact PLT-I. This is a parameter that has not been explored in the literature for this purpose.

The errors observed with the impedance method were in some cases excessive (overestimation of platelets) and in other cases deficient (underestimation of platelets). However, an underestimation could be tolerated, whereas the great constraint of an overestimation exposes the risk of missing a severe thrombocytopenia.

As a matter of fact, overestimation of platelets by impedance was reported with 24% under-transfused patients in the study of Hummel and al (3) and 96.75% of thrombocytopenic subjects in the study of Cid J and al. it was due to an overestimation of platelets by PLT-I (15). Therefore, we searched for a threshold value of MCV below which the use of PLT-O is justified.

In our study, we showed that MCV can be used as a factor pointing towards an overestimation of platelet count by the impedance technique (i.e. ΔPLT-I-PLT-O> 0). (AUC: 0.632; 95% CI: [0.568- 0.692]) and the threshold of ≤ 72.9fL has been proposed to consider PLT-O with good sensitivity.

Bavani S et al, proposed that an MCV value between 50 and 59 fL requires the use of the optical method. Indeed, the difference between this result and the value proposed by our study can be explained by the narrowness of the population studied in this Malaysian study (n=103) and the reduced number of samples with an MCV between 50 and 59 fL (n=12) (4).

In contrast, an MCV cut-off close to our own was proposed in a study conducted in Taiwan,. The authors stated that for a MCV value less than or equal to 70 fL , an overestimation is observed in the platelet count by impedance (9).

Given the scarcity of studies that have addressed this issue, no tangible conclusions can yet be drawn regarding the MCV cut-off to be adopted when deciding to use PLT-O.

Elsewhere, the cut-off of Micro R >37.3% does not seem to be effective in making this decision. Further investigations could demonstrate its value in a quality approach.

Determining a threshold for MCV below which the analyzer will automatically perform PLT-O testing could represent a new rule in the blood count validation procedure, easily integrated into cellular hematology analyzers. The aim is to provide clinicians with a reliable platelet count.

Nevertheless, our research has some limitations. Obviously, the number of patients with severe thrombocytopenia is limited. Additionally, a comparative study among different cell hematologic analyzers would be valuable to standardize decision thresholds, with this in mind, further studies are required.

Conclusion

The platelet count is a crucial diagnostic parameter with significant implications for clinical decision-making. In situations involving microcytic anemia or microcytosis, there is a risk of overestimating platelet counts through the impedance technique, potentially concealing underlying thrombocytopenia.

The findings from this study strongly suggest that the optical method outperforms the impedance method in accurately estimating platelet numbers, especially in samples with low MCV. Based on our results, impedance could be used routinely as a primary technique. However, for microcytosis below 72.9 fL, optical method could be incorporated into the blood count validation procedure for a more accurate determination of the platelet count.

Further investigations could demonstrate the value of this threshold for MCV to assess its impact on treatment decisions for patients and platelet transfusion indications.

References

1. Schved JF, Monneret I, Levayer T, Lavabre-Bertrand C, Taib J. Étude des différentes méthodes de numération plaquettaire au laboratoire.: Application aux thrombopénies sévères. Rev Fr Lab. 1999;1999(312):95-8.
2. Lakos G, Mukhtar Z, Masi L, Valente S, Papa F. Alinity hq platelet count is not impacted by severe microcytosis. J Clin Lab Anal. 2022;36(4):e24218.
3. Hummel K, Sachse M, Hoffmann JJML, van Dun LPJM. Comparative evaluation of platelet counts in two hematology analyzers and potential effects on prophylactic platelet transfusion decisions. Transfusion (Paris). 2018;58(10):2301-8.
4. Bavani s. Influence of Red Cell Microcytosis in the Accuracy of Platelet Counting by Automated Methods. Journal of Pharmaceutical Sciences and Research. 2019 [cité 11 déc 2023]; Disponible sur: https://www.jpsr.pharmainfo.in/Documents/Volumes/vol11issue04/jpsr11041964.pdf
5. Salignac S, Latger-Cannard V, Schlegel N, Lecompte TP. Platelet counting. Methods Mol Biol Clifton NJ. 2013;992:193-205.
6. Boulassel MR, Al-Farsi R, Al-Hashmi S, Al-Riyami H, Khan H, Al-Kindi S. Accuracy of Platelet Counting by Optical and Impedance Methods in Patients with Thrombocytopaenia and Microcytosis. Sultan Qaboos Univ Med J. 2015;15(4):e463-468.
7. Tantanate C. Comparison of Impedance Platelet Count by Sysmex XE-5000 and Beckman Coulter LH 780 with Optical Fluorescent Platelet Count in Thalassemia Patients. Siriraj Med J. 2017;69(5):276-82.
8. Briggs C, Harrison P, Machin SJ. Continuing developments with the automated platelet count. Int J Lab Hematol. 2007;29(2):77-91.
9. Pan LL, Chen CM, Huang WT, Sun CK. Enhanced accuracy of optical platelet counts in microcytic anemia. Lab Med. 2014;45(1):32-6.
10. Pinkowski, R. Difference between impedance and optical platelet count methods in patients with microcytosis of red blood cells. Lab Hematol 5 22-27.
11. Schoorl M, Schoorl M, Oomes J, van Pelt J. New fluorescent method (PLT-F) on Sysmex XN2000 hematology analyzer achieved higher accuracy in low platelet counting. Am J Clin Pathol. 2013;140(4):495-9.
12. Johannessen B, Haugen T, Stephen Scott C. Standardisation of platelet counting accuracy in blood banks by reference to an automated immunoplatelet procedure: comparative evaluation of Cell-Dyn CD4000 impedance and optical platelet counts. Transfus Apher Sci. 2001;25(2):93-106.
13. Tantanate C, Khowawisetsut L, Pattanapanyasat K. Performance Evaluation of Automated Impedance and Optical Fluorescence Platelet Counts Compared With International Reference Method in Patients With Thalassemia. Arch Pathol Lab Med. 2017;141(6):830-6.
14. Briggs C, Kunka S, Machin SJ. The most accurate platelet count on the Sysmex XE-2100. Optical or impedance? Clin Lab Haematol. avr 2004;26(2):157-8.
15. Cid J, Nascimento JD, Vicent A, Aguinaco R, Escoda L, Ugarriza A, et al. Evaluation of low platelet counts by optical, impedance, and CD61-immunoplatelet methods: estimation of possible inappropriate platelet transfusion. Transfusion (Paris). 2010;50(4):795-800.