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CLINICAL & BASIC RESEARCH

Accuracy of Platelet Counting by Optical and Impedance Methods in Patients with Thrombocytopaenia and Microcytosis

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الدقة في عد الصفيحات بواسطة طرق بصرية ومعاوقية عند مرضى مصابين بقلة الصفيحات وصغر الكريات الحمراء

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abstract: ***Objectives:*** Obtaining accurate platelet counts in microcytic blood samples is challenging, even with the most reliable automated haematology analysers. The CELL-DYN™ Sapphire (Abbott Laboratories, Chicago, Illinois, USA) analyser uses both optical density and electronic impedance methods for platelet counting. This study aimed to evaluate the accuracy of optical density and electrical impedance methods in determining true platelet counts in thrombocytopaenic samples with microcytosis as defined by low mean corpuscular volume (MCV) of red blood cells. Additionally, the impact of microcytosis on platelet count accuracy was evaluated. ***Methods:*** This study was carried out between February and December 2014 at the Haematology Laboratory of the Sultan Qaboos University Hospital in Muscat, Oman. Blood samples were collected and analysed from 189 patients with thrombocytopaenia and MCV values of <76 femtolitres. Platelet counts were tested using both optical and impedance methods. Stained peripheral blood films for each sample were then reviewed as a reference method to confirm platelet counts. ***Results:*** The platelet counts estimated by the impedance method were on average 30% higher than those estimated by the optical method (P <0.001). The estimated intraclass correlation coefficient was 0.52 (95% confidence interval: 0.41–0.62), indicating moderate reliability between the methods. The degree of agreement between methods ranged from -85.5 to 24.3 with an estimated bias of -30, suggesting that these methods generate different platelet results. ***Conclusion:*** The impedance method significantly overestimated platelet counts in microcytic and thrombocytopaenic blood samples. Further attention is therefore needed to improve the accuracy of platelet counts, particularly for patients with conditions associated with microcytosis.

Keywords: Electrical Impedance; Optical Devices; Platelet Counts; Thrombocytopenia; Anemia; Mean Corpuscular Volume.

الملخص: الهدف: يمثل الحصول على تعداد دقيق للصفيحات في عينات دم المصابين بصغر الكريات الحمراء تحديا كبيرا حتى عند استخدام أكثر أجهزة التحليل الأتوماتيكية وثوقية. ويقوم جهاز سيل داين CELL-DYN™ (من معامل أبوت بشيكاغو–ولاية الينوي–الولايات المتحدة) باستخدام الكثافة البصرية والمعاوقية الكهربائية لعد الصفيحات. ويهدف هذا البحث لتقويم دقة الطرق البصرية والمعاوقية الكهربائية لتحديد أعداد الصفيحات بدقة في عينات دماء المرضى المصابين بقلة الصفيحات وصغر الكريات الحمراء، والمعرفة بصغر حجم الكرية الوسطى (MCV). وتم كذلك بحث تأثير صغر الكريات الحمراء على دقة عد الصفيحات. **الطريقة:** أجريت الدراسة بين فبراير وديسمبر 2014م. بمختبر علم الدم بمستشفى جامعة السلطان قابوس بمسقط في عمان. وجمع عينات دم من 189 من المرضى المصابين بقلة الصفيحات وقيم MCV أقل من 76< فيمتوليتر. وتم عد الصفيحات عن طريق استخدام الكثافة البصرية والمعاوقية الكهربائية. وتم فحص شرائح دم محيطي من كل عينة دم كمرجعية لعد الصفيحات. **النتائج:** كانت نتائج عد الصفيحات بواسطة الطريقة المعاوقية تفوق تلك المتحصل عليها من بالطريقة البصرية بنحو 30% في المتوسط (P <0.001) وكان معامل الارتباط هو 0.52 (95% فاصل الموثوقية: 0.41–0.62) مما يؤشر إلى اعتمادية متوسطة عند الطريقتين. وتراوحت درجة التوافق بين الطريقتين بين 85.5- و 24.3، مع تحيز (خطأ منهجي) يبلغ 30-. مما يدل على أن الطريقتين تخرجان بنتائج مختلفة لعدد الصفيحات. **الخلاصة:** النتائج المتحصل عليها من الطريقة المعاوقية هي أكبر من غيرها عند استخدامها في دماء مرضى مصابين بقلة الصفيحات وصغر الكريات الحمراء. لذا ينبغي الاهتمام أكثر بتحسين الدقة، خاصة عند المرضى المصابين بصغر الكريات الحمراء.

مفتاح الكلمات: الطريقة المعاوقية الكهربائية؛ الأجهزة البصرية؛ عد الصفيحات؛ قلة الصفيحات؛ الأنيميا؛ حجم الكرية الوسطى.

Advances in Knowledge

- *The findings of this study indicate that the impedance method overestimates the platelet count in thrombocytopaenic samples with low mean corpuscular volume (MCV) of red blood cells in comparison to the optical method. The optical method is therefore more reliable for platelet counting in samples with a low MCV value and thrombocytopaenia.*

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Application to Patient Care

- *Physicians and laboratory scientists should keep in mind that the impedance method may overestimate the platelet count in samples with microcytosis and thrombocytopaenia, thereby potentially affecting transfusion decisions for patients at risk of bleeding.*
- *The results of the study indicate that more attention needs to be directed towards improving the accuracy of platelet counts, particularly for patients with conditions associated with microcytosis and thrombocytopaenia.*

In most clinical laboratories, platelet counts are routinely and reliably performed by modern automated blood cell analysers. However, the lack of accuracy of automated analysers when enumerating low platelet counts continues to pose problems.[1] Recent studies have shown significant inaccuracies occurring among current automated haematology analysers when counting platelets at low levels; these may subsequently lead to the provision of over- or under-transfusions of platelet concentrates to patients at risk of bleeding.[2–4] These findings are concerning because transfusion decisions based on inaccurate platelet counts may either result in serious bleeding complications or waste valuable blood products. Furthermore, patients may be unnecessarily exposed to blood products and their associated complications. Finding a reliable method to enumerate low platelet counts therefore remains a challenge. Currently, two basic methods—optical density and electrical impedance—are employed by automated haematology analysers to count platelets.[1] An optical platelet count is generally obtained through a two-dimensional analysis that estimates the complexity and density of platelets represented as a cytogram of the light intensity at 7° and 90° angles. The impedance platelet count uses hydrodynamic focusing and single-dimensional histogram analysis to count the platelets based on their size.

While most automated analysers use either method separately, newer equipment can now use both optical and impedance techniques simultaneously, for example the CELL-DYN™ Sapphire (Abbott Laboratories, Chicago, Illinois, USA) and the XE-2100™ (Sysmex Corp., Kobe, Japan) analysers. However, both methods have been associated with limitations that may affect the accuracy of the platelet count. These limitations are mainly related to the inability of automated analysers to discriminate between platelet and non-platelet particles, such as microcytic or fragmented red blood cells, cell debris, white cell fragments and giant platelets.[1] To overcome these limitations, immunological methods using flow cytometry technology have been developed that use conjugated monoclonal antibodies directed against specific platelet antigens such as cluster of differentiation (CD) 41 and CD61.[1,5] Although immunological methods are highly accurate for counting platelets even in severely thrombocytopaenic samples, they are not available in all laboratories and the total cost per platelet count is expensive compared to the optical or impedance methods.[1]

Limited information is available on the accuracy of optical and impedance methods for platelet counting in cases of thrombocytopaenia with microcytosis, as defined by the mean corpuscular volume (MCV) of red blood cells. As microcytosis can lead to inaccurate platelet counting by automated analysers, determining true platelet counts is necessary to minimise counting errors, especially in areas where causes of microcytosis are common, such as thalassaemia and iron deficiency anaemia, as both of these conditions are associated with low MCV.[6] Reliably determining the true platelet count in these conditions may help not only the physicians in charge of care, but also health providers and laboratory scientists to optimise workflow and meet the demands of increasing workloads. To date, the accuracy of platelet counts by either the impedance or optical method is questionable, particularly in thrombocytopaenic samples due to chemotherapy, bone marrow transplantation or marrow diseases associated with myeloid aplasia or myelodysplasia. Indeed, the accuracy of both methods is further influenced by some conditions affecting the size of red blood cells that result in low MCV, thereby leading to false platelet counts. Therefore, this study aimed to evaluate the optical and impedance methods for platelet counting in thrombocytopaenic samples with microcytosis from hospitalised adult patients in Oman. Platelet counts produced by the two methods were compared with those obtained from a microscopic examination of blood smears as a reference method. Additionally, the study aimed to assess the impact of microcytosis on platelet count accuracy.

Methods

This study was carried out between February and December 2014 at the Haematology Laboratory of the Sultan Qaboos University Hospital in Muscat, Oman. Blood specimens were collected from the morning batch of samples received at the laboratory for complete blood counts. Samples selected for inclusion in the study were those with a platelet count of <100 x 10^9/L (normal range: 150–450 x 10^9/L), indicating

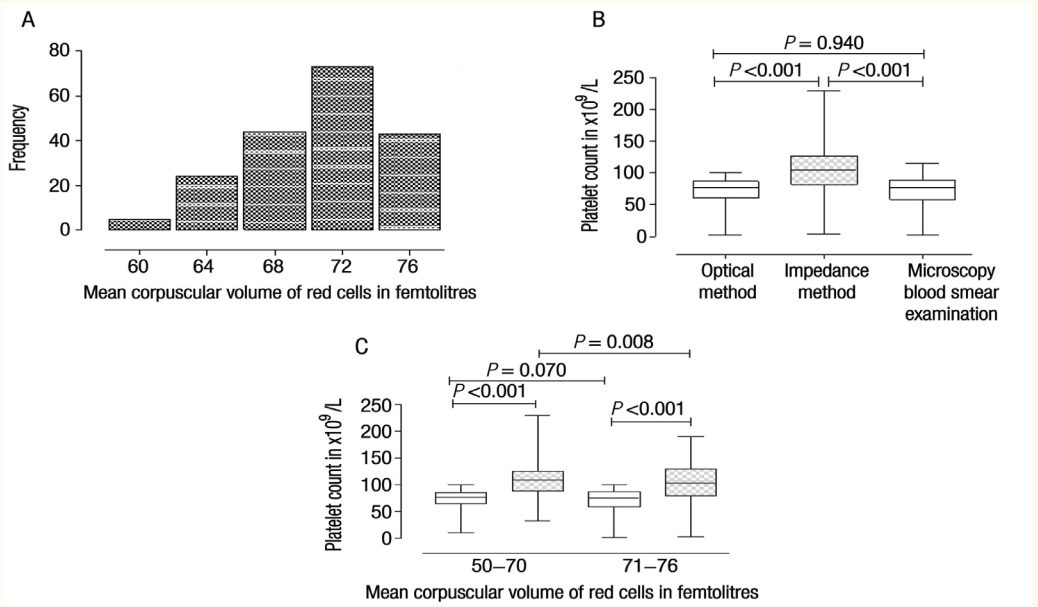

Figure 1A–C: Distribution of mean corpuscular volume (MCV) of red blood cells and platelet counts in thrombocytopaenic blood specimens with microcytosis (N = 189). **A:** Frequency of MCV distribution in the study population. **B:** Comparison of platelet counts among the optical method, impedance method and microscopic examination of blood smears. The minimum, maximum, median and *P* values for each boxplot are shown. **C:** Comparisons of platelet counts between the optical and impedance methods according to MCV values. The minimum, maximum, median and *P* values for each boxplot are shown. The open and filled boxes represent the optical and impedance methods, respectively.

thrombocytopaenia, and those with a MCV of ≤76 femtolitres (fL) (normal range: 78–95 fL), indicating microcytosis. Samples with MCV values of 61–70 fL constituted group one while samples with values of 71–76 fL constituted group two. The patients' specific diseases or conditions were not considered in the inclusion or exclusion criteria.

All blood samples were collected in ethylenediaminetetraacetic acid tubes and were tested four to six hours after the phlebotomy. The CELL-DYN™ Sapphire (Abbott Laboratories) analyser was used to enumerate platelet counts by both optical and impedance methods. Calibration, quality control and maintenance procedures were performed daily according to the manufacturer's instructions. Floating thresholds were used to discriminate between platelets and non-platelet particles. Optical and impedance platelet counts were measured independently on each blood sample. Stained peripheral blood smears were also evaluated by microscopy to obtain a reference platelet count against which to evaluate the accuracy of the optical and impedance methods. Furthermore, blood smears were evaluated to determine the presence of factors that could interfere with platelet counts (including platelet aggregates, thrombocyte abnormalities, cell debris and white and red blood cell fragments).

Analyses were performed using GraphPad Prism, Version 5 (GraphPad Software, Inc., San Diego, California, USA). Descriptive statistics for platelet counts were produced for each method. The paired Student's t-test and Pearson's correlation coefficient were used to evaluate the difference in mean platelet counts and to measure the linear regression between the methods, respectively. The Bland-Altman method was used to assess agreement between measurements. The reliability of the platelet measurements was evaluated by the intraclass correlation coefficient (ICC). The patients were divided into two groups according to MCV values and the mean value of the overall data in each group was used to examine the influence of MCV on platelet counts for each method. All tests were two-tailed with an alpha level of 0.05.

This study was approved by the Medical Research & Ethics Committee at the College of Medicine & Health Sciences of Sultan Qaboos University, Muscat, Oman (MREC #680).

Results

A total of 189 thrombocytopaenic and microcytic blood specimens were included in the study. The examined study population were between 17 and 85 years old with a mean age of 39 ± 18 years. Of the patients who contributed these samples, 88 (47%) were male. According to the optical method, platelet counts ranged from 3–100 x 10^9/L with a mean count of 73 ± 19 x 10^9/L. The mean MCV of red blood cells was 70 ± 4 fL (range: 61–76 fL) [Figure 1A]. The mean red cell distribution width was 18 ± 4% (range: 12–34%).

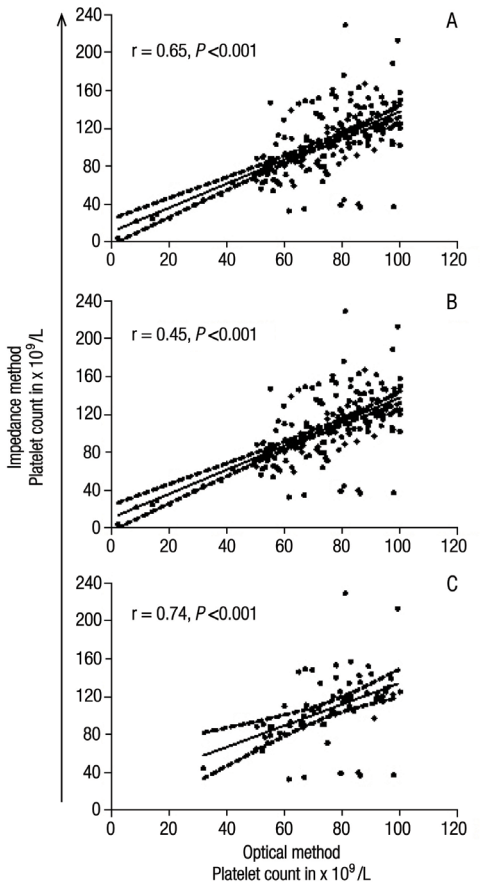

Figure 2A–C: Linear regression analyses of platelet counts between optical and impedance methods in thrombocytopaenic blood specimens with microcytosis (N = 189). The dotted lines show the 95% confidence band of the best-fit line. The correlation coefficient and *P* values are shown. **A:** Correlation analysis of platelet counts using the entire data set. **B:** Correlation analysis of platelet counts using samples with mean corpuscular volume (MCV) values of 60–70 femtolitres (fL). **C:** Correlation analysis of platelet counts using samples with MCV values of 71–76 fL.

The impedance method failed to provide counts for two samples that had low platelet counts (<10 x 10^9/L) according to the optical method. The impedance method yielded significantly higher platelet counts when compared to the optical method

Table 1: Reliability analysis for platelet counts by optical and impedance methods in thrombocytopaenic blood specimens with microcytosis (N = 189)

	Intraclass correlation coefficient	95% confidence interval		*P* value
		Lower bound	Upper bound	
Single measure	0.52	0.41	0.62	<0.001
Average measure	0.68	0.58	0.76	<0.001

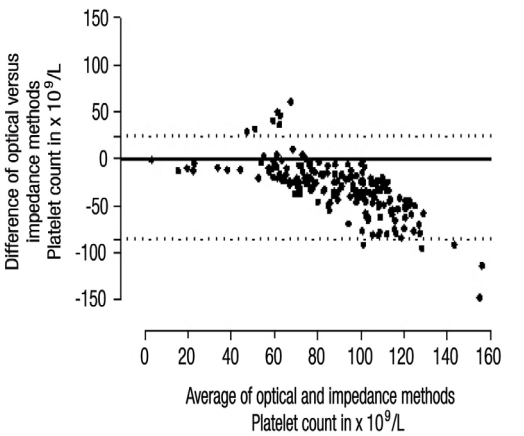

Figure 3: Bland-Altman plot depicting the degree of agreement between optical and impedance methods in platelet counts among thrombocytopaenic blood specimens with microcytosis (N = 189). The solid line represents zero difference between the two methods. The dotted lines represent the upper and lower limits of agreement between the two methods as measured by the mean ± two standard deviations.

or the microscopy examination of blood smears (*P* <0.001) [Figure 1B]. Estimated platelet values from the impedance method were on average 30% higher than those of the optical method. However, in five samples, the impedance method measured a platelet count that was two-fold lower than that observed by the optical method. Microscopic examination of the peripheral blood smears revealed no platelet clumps, giant platelets or white or red blood cell fragments in the majority of cases (92%). Additionally, microcytosis was evident on all blood films, supporting the MCV values obtained by the complete blood count.

The samples were divided into two groups according to mean MCV values. The impedance method showed significantly higher platelet counts in both groups compared to the optical method [Figure 1C]. Interestingly, no significant difference was found between the groups for platelet counts assessed by the optical method (74 ± 16 x 10^9/L versus 72 ± 20 x 10^9/L; *P* = 0.070). In contrast, a significant difference was observed between the two groups for platelet counts assessed by the impedance method (107 ± 4 x 10^9/L versus 102 ± 3 x 10^9/L; *P* = 0.008).

Linear regression analysis revealed a moderately positive correlation between optical and impedance methods (r = 0.65; *P* <0.001) [Figure 2A]. Interestingly, this correlation, although statistically significant, was weaker (n = 73; r = 0.45; *P* <0.001) in samples with lower MCV values [Figure 2B] and stronger (n = 116; r = 0.74; *P* <0.001) in samples with higher MCV values [Figure 2C]. The estimated ICC was 0.52 (95% confidence interval: 0.41–0.62), indicating moderate

to fair reliability between the two methods [Table 1]. The degree of agreement between the two methods was also in line with these findings [Figure 3]. The estimated bias was -30 with a reasonably wide limit of agreement ranging from -85.5 to 24.3.

Discussion

There remains some debate regarding which method is most accurate for platelet counting in thrombocytopaenic samples with microcytosis. While some studies report that the optical method is more accurate in assessing samples with low platelet levels, others have shown that the impedance method gives the best platelet count in chemotherapy samples.[1,7,8] So far, few studies have investigated the accuracy of platelet counting in thrombocytopaenic samples with microcytosis, particularly in regions with a high prevalence of thalassaemia carriers, such as Oman, where the α-thalassaemia gene is seen in 48% of the local population.[6,9]

The current study found that the impedance method yielded a higher platelet count compared to the optical method and the reference method (microscopic examination of blood smears). These results were in agreement with those reported by Pińkowski, who also found that the optical method yielded a more reliable platelet count in microcytic samples than the impedance method.[10] However, the two studies differed in many aspects. Firstly, in Pińkowski's study, 90% of the 30 microcytic blood samples showed a normal platelet count of above 150 x 10^9/L by the optical method,[10] while all 189 samples in the present study had thrombocytopaenia with platelet counts below 100 x 10^9/L. Secondly, the mean MCV value in Pińkowski's study was 73 ± 5.9 fL (range: 57–80 fL) and 43% of the samples had MCV values >76 fL.[10] In comparison, all samples in the present study had MCV values <76 fL. Lastly, different instruments were used between the studies, with the CELL-DYN™ 4000 (Abbott Laboratories) automated analyser being used in Pińkowski's study,[10] in comparison to the CELL-DYN™ Sapphire (Abbott Laboratories) analyser utilised in the present study. Nevertheless, it should be noted that both blood cell analysers rely on the same optical and impedance principles for platelet counting.

Interestingly, the impedance method further overestimated platelet counts in samples with MCV values <70 fL in the current study. In addition, it showed a weak but significant correlation with the optical method. These findings suggest that the impedance method will not provide the most accurate platelet counts at low MCV values and may instead yield a false-high count, potentially affecting platelet transfusion decisions for patients. These results are in line with those from a study by Ninama *et al.*, who reported that the impedance method was not always reliable for assessing platelet counts in cases of severe microcytosis after comparing platelet counts obtained by the impedance method on the CELL-DYN™ 3700 (Abbott Laboratories) analyser with those obtained from a manual technique with ammonium oxalate.[11] Similarly, Pan *et al*. showed that the impedance method overestimated platelet counts in microcytic samples using the XE 2100™ automated analyser (Sysmex Corp.).[12] Collectively, these findings suggest that the optical method is more accurate for estimating platelet counts in samples with low MCV values, especially in those with severe microcytosis. However, it should be noted that five samples assessed by the impedance method in the current study showed a platelet count that was two-fold lower than that indicated by the optical method. In these samples, a review of the peripheral blood films showed that the true platelet count was close to the values yielded by the optical method. It is of concern that the true platelet counts in these five cases did not correspond with the general trend of overestimation seen with the impedance method.

Although the comparison of the two methods in the current study demonstrated a moderately positive correlation, this does not necessarily mean that the techniques are interchangeable. The same association was not observed with the ICC value, which determines the reliability of the impedance method to yield the same or compatible platelet counts in comparison to the optical method. The ICC is calculated using variance estimates, which are obtained from the analysis of platelet measurement variance, and is measured on a scale from 0 to 1, where the closer the value is to 1, the higher the reliability. Excellent reliability is usually determined by an ICC value of ≥0.75, which was not obtained in the current study.[13] Additionally, the Bland-Atman limits of agreement, which assume that differences are constant throughout the range of platelet measurements, further indicate that the impedance method produced different platelet counts with a high level of bias in comparison to the optical method.

The current study had a number of limitations. First, selected blood samples were tested within four to six hours following the phlebotomy. It is possible that this may have caused the platelets to swell when measured by the impedance method or the internal intensity of platelets to decrease when using the optical method. Second, blood samples with normal MCV values and platelet counts were not included as a

control group. Finally, immunological-based methods of counting platelets were not used.

Conclusion

The results of the current study provide evidence that the optical method is superior to the impedance method in estimating platelet counts in samples with low MCV values. As a result, physicians and laboratory scientists should keep in mind that the impedance method may significantly overestimate the platelet count in microcytic samples with thrombocytopaenia, which may potentially affect transfusion decisions. More attention needs to be directed towards improving the accuracy of platelet counts, particularly for patients with conditions associated with microcytosis.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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