Use of Mindray MC-80 digital morphology analyzer's estimated platelet counts as adjunct to automated hematology analyzer

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Abstract

Introduction: Automated hematology analyzer platelet counts can be performed using either the impedance (PLT-I) method or the fluorescent nucleic acid staining (PLT-O) method. Estimated platelet counts (PLT-E) can be calculated using a digital morphology analyzer by evaluating the peripheral blood smear. Our objective was to compare the platelet values detected on a Mindray BC-6200 device to the PLT-E count on a Mindray MC-80 digital morphology analyzer.

Material and methods: Complete blood cell count findings between 1 September and 11 October, 2022 were obtained from the data storage units of the devices. We selected two groups of blood: a first group with thrombocytopenia (n = 49) and a second group that gave an aggregation and/or platelet clumping flag (n = 32). The results of 190 consecutive patients with normal platelet counts, and no aggregation flag, were evaluated as a control group. Pearson's correlation coefficients, Bland-Altman plots, and paired t-tests were calculated.

Results: The plot of the difference between PLT-I and PLT-O counts showed that the mean difference was –43.6 (95%: –17.2 to –69.9); when we compared PLT-O to PLT-E, bias was improved to –6.1 (95%: –18.26 to 6.1) in samples with aggregation and/or clumping flags (Bland-Altman plots).

In samples with thrombocytopenia without aggregation and/or clumping, on the Bland-Altman plot, the differences in means were all close to zero, and there were no definite biases.

Conclusions: Examining blood samples using the Mindray MC-80 digital morphology analyzer system on samples that show platelet clumps has the potential to improve PLT-I results in day-to-day laboratory routine.

Key words: method comparison, platelet estimation, digital morphology analyzer, thrombocytopenia, auto analyzer

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Introduction

The main function of platelets is to maintain vascular integrity after injury to the vascular system. In addition to hemostasis and thrombosis, platelets also play an important role in the biology of inflammation, immunity, and cancer [1].

Accurately determining the platelet number is of the utmost importance because a deficiency can cause bleeding [2]. However, conditions such as cold platelet agglutinins, paraproteinemias, platelets coming into contact with foreign surfaces (such as a dialysis membrane), giant platelets, hyperlipemia, platelet aggregation due to ethylenediaminetetraacetic acid (EDTA), or overfilled blood samples can also cause spuriously low platelet counts incompatible with patient's clinics (pseudothrombocytopenia) [2, 3]. Pseudothrombocytopenia has been reported in patients suffering from various conditions and, more recently, in patients with coronavirus disease 2019 (COVID-19) [4, 5]. Pseudothrombocytopenia can lead to misdiagnosis and, ultimately, the selection of inappropriate treatments for the patient [4, 6].

In automated hematology analyzers, platelets are counted as part of the complete blood cell count (CBC) by impedance, optical methods (light diffraction or fluorescence techniques), and immunofluorescence techniques using monoclonal antibodies directed against glycoproteins of the surface membrane of platelets [2]. Automated hematology analyzers can produce accurate CBC results for virtually any sample. However, from time to time, inaccurate results may occur [7]. Flags or messages from the devices regarding these 'spurious' changes differ depending on the analyzer and the method [8].

With impedance-type instruments, platelet and red blood cell count (RBC), which are both analyzed in the same channel(s), are discriminated according to their volume, and volume histograms are then generated. With the Mindray BC-6200, platelet counts as a part of CBC can be done in two ways: by platelet counts based on the DC sheath-flow impedance (PLT-I) method (based on DC sheath-flow impedance), or by platelet counts based on fluorescent nucleic acid staining and done in the reticulocyte channel (PLT-O) method. If there is a 'platelet aggregation' or a 'low platelet count' alarm from the hematology analyzer, a reflex test can be performed using the PLT-O method. It has been claimed that PLT-O detection technology can effectively correct platelet counts [9].

In routine laboratory practice, microscopic examination of peripheral blood smear slides by a trained specialist is required for thrombocytopenic samples. However, manually examining the platelet count is time-consuming and labor-intensive. Recent advances have allowed platelet count estimation using digital morphology analyzer imagery and software algorithms [10]. These increase efficiency and reduce inconsistency between observers, especially in laboratories with a large number of patient samples.

Our objective was to compare the PLT-I and PLT-O values detected by the Mindray BC-6200 device to the MC-80 digital morphology analyzers' estimated platelet counts (PLT-E) test results, and to determine its efficacy for the confirmation of pseudothrombocytopenia.

Material and methods

This was a retrospective data analysis study. The study protocol was approved by the Ethics Committee of the Institution (2011-KAEK-25 2022/11-10).

CBC findings between 1 September and 11 October, 2022 were obtained from the data storage units of the devices. In our laboratory, blood is taken into K3-EDTA-containing vacutainer tubes (Ayset Medical Products, Adana, Turkey) for CBC analysis. According to laboratory protocol, CBC analysis is completed within two hours after blood collection. During this period, a total of 59,856 hemograms were studied in our laboratory, including from outpatients, inpatients, and asymptomatic individuals who came to the hospital only for the purpose of health screening.

Platelet counts (as a part of the CBC) were performed using an automated hematology analyzer, the Mindray BC-6200 (Mindray, Shenzhen, China). Internal quality control tests were performed in accordance with the manufacturer's instructions. External quality control was performed in accordance with KBUDEK (External Quality Control Program, Istanbul, Turkey).

In our laboratory routine, PLT-I results were reported after the first measurement. Once the samples with a thrombocytopenia and/or platelet aggregation flag were detected, they went under the verification protocol for PLTs, which includes opening the reticulocyte channel. The result that was analyzed in the PLT-O mode by staining with a specific fluorescent dye with high specificity and sensitivity was reported at the second measurement. At the same time, peripheral blood smear (PBS) staining and examination were also performed [9, 11].

SC120 automated slidemakers (Mindray, Shenzhen, China) were used to prepare PBSs stained with Wright-Geimsa dye [12]. PBSs were evaluated using an MC-80 digital morphology analyzer. The analyzer classifies white blood cells (WBCs) and red blood cells (RBCs) and include functionality for estimation of platelet count. For our analysis, only the platelet count data was used.

Statistical analysis

Statistical analysis was performed using MedCalc® Statistical Software version 20.121 (MedCalc Software Ltd, Ostend, Belgium) and SPSS version 14.0 (SPSS Inc., Chicago, IL, USA).

Table I. Mindray BC-6200 automated hematology analyzer complete blood count platelet indices analyzed usingimpedance method

Parameter	Group 1*	Group 2**	Control
N	49	32	190
PLT-I count, mean ± SD [× 10⁹/L]	49.5 ± 34.2	75.0 ± 33.3	289.8 ± 77.4
PLT-O count, mean ± SD [× 10⁹/L]	45.9 ± 33.5	112.5 ± 71.0	
PLT-E count, mean ± SD [× 10⁹/L]	44.7 ± 32.0	115.1 ± 68.3	270.8 ± 77.6
PDW [%]	16.7 ± 1.12	16.5 ± 1.01	16.1 ± 0.30
MPV [fL]	11.7 ± 1.8	12.7 ± 1.9	11.4 ± 1.0

*Thrombocytopenia; **aggregation/clumping flag; N — number of patients' samples; PLT — platelet; PLT-I — impedance method; SD — standard deviation; PLT-O — fluorescent nucleic acid staining method; PLT-E — calculated using a digital morphology analyzer; PDW — platelet distribution width; MPV — mean platelet volume; fL — femtoliters

The mean ± standard deviation (SD) and median values of the variables were calculated. One-way repeated measures analysis of variance (ANOVA) was used to determine significant differences between the related means. We performed a paired sample t-test or aWilcoxon matched paired test where appropriate (depending on the distribution of the measurements) to determine the significance of the differences between groups. Agreement between assays were evaluated by Passing-Bablok regression analysis, Spearman's rank correlation coefficient (rho), and Bland-Altman plots. A p value ≤0.05 was considered statistically significant.

Results

We selected two groups of blood specimens with a PLT-I level ranging from $2 × 10^9$/L to $146 × 10^9$/L: the first group with thrombocytopenia, and the second group that gave an aggregation and/or platelet clumping flag using a Mindray BC-6200 automated hematology analyzer and/or an MC-80 digital morphology analyzer. The results of 190 consecutive patients with normal platelet counts ($158 × 10^9$/L to $448 × 10^9$/L) and no aggregation flag were evaluated as a control group.

During the study period, the results of the blood specimens, a total of 81 specimens from CBC with thrombocytopenia and/or platelet aggregation/clumping flag, were evaluated. The age range of patients was 1–94 years. Twenty-two patients were followed up for malignancy: five for idiopathic thrombocytopenic purpura, five for chronic renal failure, six for chronic viral hepatitis, four for connective tissue disease, and the remaining two patients with various diagnoses.

Of the patient samples, one with thrombocytopenia and another one with an aggregation flag were analyzed 10 times with the Mindray BC-6200 device in PLT-I and PLT-O modes, and calculated CVs were found to be acceptable, in the range 2.58–4.61% [13].

The thrombocytopenic samples without aggregation and/or clumping flag from the Mindray BC-6200 analyzer and/or Mindray MC-80 (n = 49) had a mean platelet level of $49.5 ± 34.2 × 10^9$/L and a median platelet level of $46.0 × 10^9$/L [interquartile range (IQR): 65] in PLT-I mode. The CBC was reanalyzed in the reticulocyte channel PLT-O, and the mean PLT count was $45.9 ± 33.5$ with a median count of $43.0 × 10^9$/L (IQR: 54). The PLT-E was $44.7 ± 32.0 × 10^9$/L with a median of $36 × 10^9$/L (IQR: 59) using the MC-80 system (Table I). One-way repeated measures analysis of variance (ANOVA) showed no significant group difference between the methods (F = 2.83, p = 0.064).

After reanalyzing thrombocytopenic samples without aggregation/clumping flags, PLT-E values with PLT-O and with PLT-I were strongly correlated, Spearman's rho values were 0.914 and 0.902, respectively (p <0.001, p <0.001).

In samples with thrombocytopenia without aggregation and/or clumping, the plot of the difference between the automated analyzer Mindray BC-6200's PLT-O and PLT-I count values against their means according to the Bland-Altman design showed that the difference in means was 3.6 (95% CI: 0.04–7.18; Figure 1). On the Bland-Altman plot, the differences in means were all close to zero, and there were no definite biases, although outliers were identified.

In samples from the aggregation and/or clumping group, 11 gave the aggregation flag using the Mindray BC-6200 analyzer, 24 gave the clumping flag using the MC-80 analyzer, and six gave flags from both analyzers.

The mean platelet level of 32 patients with aggregation and/or clumping flags using the Mindray BC-6200 analyzer and/or MC-80 was $75.0 ± 33.3 × 10^9$/L, with a median of $72.5 × 10^9$/L (IQR: 51.2) in PLT-I mode. The reanalyzed PLT-O mean in the reticulocyte channel of the patients was $112.5 ± 71.0 × 10^9$/L, and the median was $97 × 10^9$/L (IQR: 104.7). With the MC-80 system, the PLT-E mean was $115.1 ± 68.3 × 10^9$/L, with a median of $92.5 × 10^9$/L (IQR: 123.5). However, the platelet counts were still lower than $100 × 10^9$/L with 18 specimens, although these counts had increased upon reevaluation.

One-way repeated measure analysis of variance (ANOVA) showed a significant difference between the methods (F = 11.7, p <0.001). The concentrations obtained by the PLT-I method were lower than those obtained by the PLT-E and PLT-O methods (p <0.001 and p = 0.002, respectively).
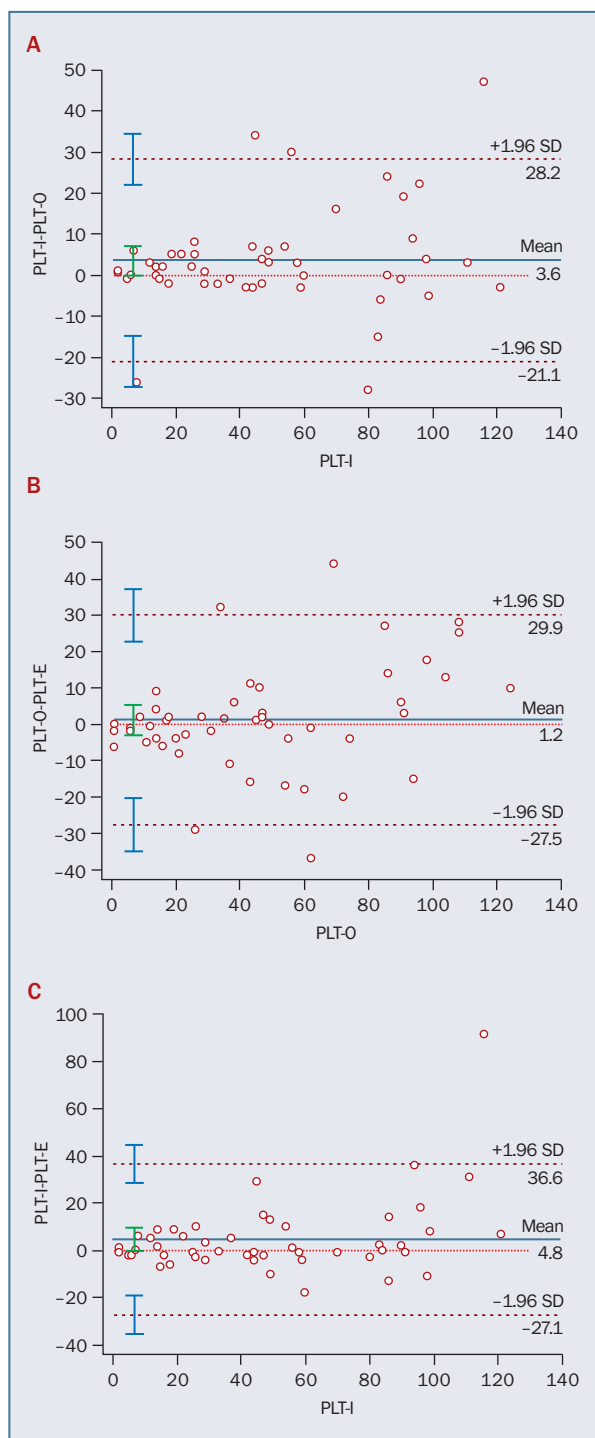
Figure 1. In samples with thrombocytopenia without aggregation and/or clumping, plot of difference between: **A.** Platelet counts based on DC sheath-flow impedance method (PLT-I) and platelet counts based on fluorescent nucleic acid staining and done in reticulocyte channel (PLT-O) values against their means according to Bland-Altman design shows that difference in means was 3.6 [95% confidence interval (CI): −0.04–7.18]; **B.** PLT-O and estimated platelet counts (PLT-E) values against their means was 4.8 (95% CI: −0.08–9.42); **C.** PLT-I and PLT-E count values against their means was 1.18 (95% CI: −3.02–5.39). Outer solid lines are upper and lower limits of agreement; SD — standard deviation

The concentrations obtained by the PLT-E and PLT-O methods were not statistically different (p = 0.318).

Regression analysis of samples with aggregation and//or clumping flags using the Mindray BC-6200 analyzer and/or MC-80 between PLT-O and PLT-I was rho = 0.367, p = 0.039 (Figure 2), and for PLT-I with PLT-E was rho = = 0.157, p = 0.391. A comparison of the digital morphology analyzer system PLT-E count to the PLT-O count showed a rho value of 0.807 (p <0.001). For samples with aggregation/clumping flags, the plot of the difference between the PLT-I and PLT-O counts against their means according to the Bland-Altman design showed that the mean difference was −43.6 (95% CI: −17.2 to −69.9) (Figure 3), and when we compared PLT-O to PLT-E, bias was improved to −6.1 (95% CI: −18.26 to 6.1) (Figure 3).

The platelet count values were obtained by the PLT-I and PLT-E values on the same blood samples of the control samples respectively (Table I).

Discussion

We found that platelet counts with PLT-I and repeat-test PLT-O modes using the Mindray BC-6200 analyzer's and morphology analyzer's PLT-E values of samples with thrombocytopenia without aggregation were close to each other.

However, in samples with aggregation and/or clumping flags from the Mindray BC-6200 and/or MC-80, the results of PLT-I and PLT-O counts were quite different from each other.

Clinically, a platelet count below 100×10^9/L indicates the risk of bleeding [14]. Using the repeat tests, the aggregation- and/or clumping-related outcomes of eight patients' platelet values changed significantly from high-risk bleeding.

Most clinical laboratories use the impedance technique for platelet counting, which involves changing the density of an electrical current as the blood particle passes through two electrodes. However, this method has some limitations [15]. It does not distinguish platelets from other blood elements with similar size ranges, which is highly imprecise in various clinical situations, despite the application of computerized algorithms [16]. Interferences such as platelet aggregation and giant platelets can cause a false decrease, and fragments of red and white blood cells can cause a false increase, in platelet counts using the PLT-I method [16].

While the impedance method (PLT-I) is generally used in routine settings, PLT-O is established as a 'reflex test' in laboratories when thrombocytopenia or an aggregation flag is found. More importantly, it has recently been described as an effective method for correcting falsely low platelet counts [11, 13].

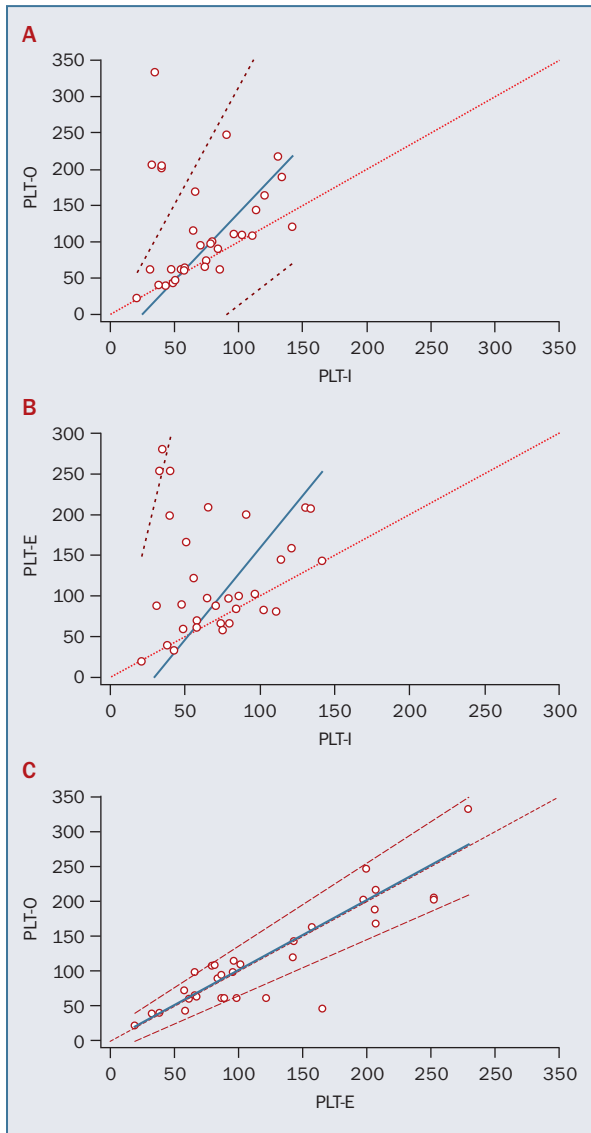While PLT-I and PLT-O results were different in the samples with aggregation, the PLT-E values obtained by

Figure 2. Regression analysis of samples with aggregation and/or clumping flags using Mindray BC-6200 analyzer and/or MC-80 between: **A.** Platelet counts based on fluorescent nucleic acid staining and done in reticulocyte channel (PLT-O) and platelet counts based on impedance method (PLT-I) (rho = 0.367); **B.** MC-80 digital morphology analyzer's estimated platelet counts (PLT-E) and PLT-I counts (rho = 0.157); **C.** PLT-E and PLT-O counts (rho = 0.807). Confidence intervals for regression lines are dashed

Figure 3. In samples with aggregation and/or clumping flags, plot of difference between: **A.** Platelet counts based on impedance method (PLT-I) and platelet counts based on fluorescent nucleic acid staining and done in reticulocyte channel (PLT-O) values against their means according to Bland-Altman design shows that difference in means was −43.6 (95%: −69.9 to −17.2); **B.** PLT-I and PLT-E count values against their means was −49.6 (95%: −76.5 to −22.8); **C.** PLT-O and MC-80 digital morphology analyzer's estimated platelet counts (PLT-E) values against their means was −6.1 (95%: −18.3 to 6.1). Outer solid lines are upper and lower limits of agreement; SD — standard deviation

evaluating and calculating stained preparations with a digital morphology analyzer and PLT-O results were correlated with each other, and the bias was quite low. Manual microscopy has been reported to be a more specific option for rechecking platelet counts. However, the manual microscopy method also has many limitations: the test is difficult, time consuming, and has low reproducibility [9]. The newly introduced digital morphology analyzer method, on the other hand, is automated, has good reproducibility,
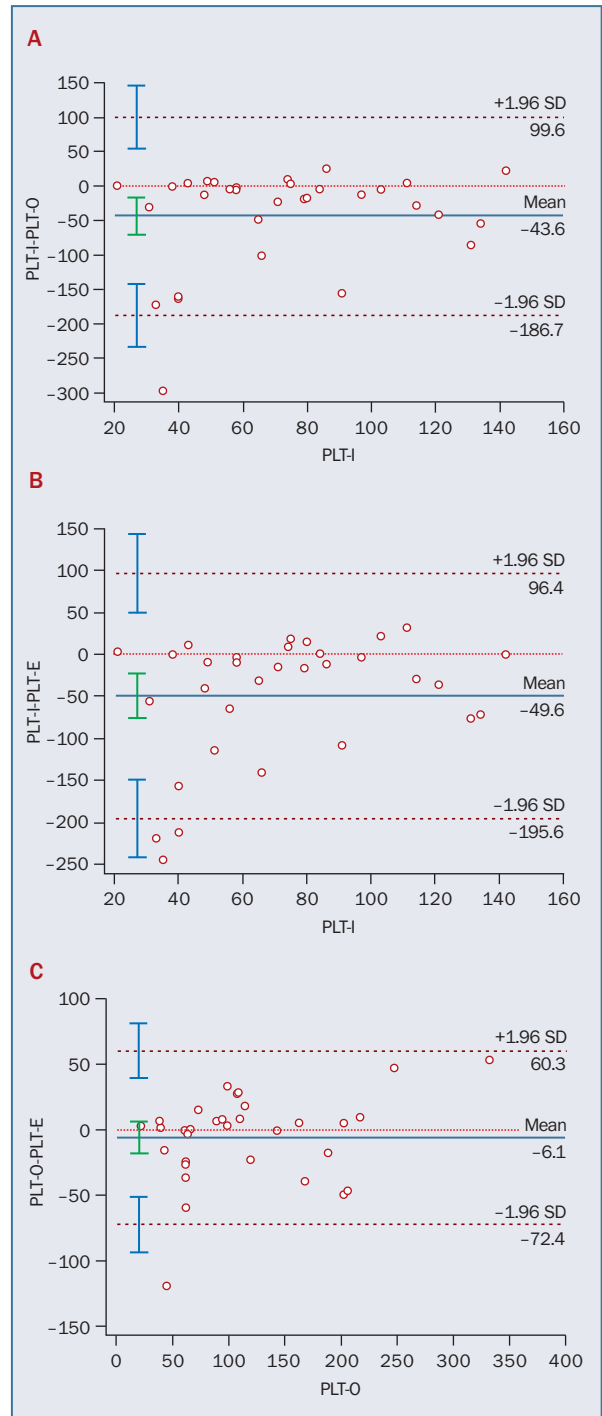
and allows discussion between clinicians regardless of their physical location.

In recent years, several automated digital morphology analyzer systems, such as the Cobas m511, CellaVision systems and Sysmex systems, have been developed for use in medical laboratories [17]. Gao et al. [18] compared platelet counts obtained using the CellaVision DM96 system (CCS; CellaVision, Lund, Sweden) to automated hematology analyzers (Beckman Counter LH 780 or Unicel DXH 800 analyzers). They found that the two analyzers showed good compatibility with manual platelet estimates. Kim et al. [19] observed that the DI-60 system (Sysmex, Kobe, Japan) and the Sysmex XN hematology analyzer had results comparable to each other for PLT estimation, but that there was a tendency to underestimate platelet counts in samples with marked thrombocytosis. However, to the best of our knowledge, this has never been evaluated in the presence of platelet clumps. With the continuing development of artificial intelligence methods, digital microscopes in our labs in the near future will replace manual processes, just as automatic urine analyzers are replacing manual urine microscopy [20]. Reliable platelet counts from blood smear images using image processing techniques will increase standardization and efficiency by reducing laboratory staff involvement [21, 22].

Limitations

This study has several limitations. Firstly, we did not confirm the platelet count using the immunological platelet counting method [23, 24]. Secondly, since the specificity and sensitivity of platelet-related alarms/flags vary widely, and any abnormal distribution in the platelet curve from the CBC result from the hematology analyzer should be examined, we did not evaluate that. There were no peer-reviewed manuscripts whether platelet count validation with the MC-80 digital morphology analyzer was performed by the manufacturer.

Further well-designed studies with a large number of participants are needed to demonstrate whether the combined use of a digital morphology analyzer and the Mindray PLT-O method achieves the most accurate reporting of spuriously low platelet counts. Further investigations should be performed, including the necessary verification of the results obtained by the MC-80 digital morphology analyzer in combination with the reference method.

Conclusions

Laboratory professionals should recognize unreliable results and identify possible causes. Evaluation of a decreased platelet count to rule out the presence of pseudothrombocytopenia, thus avoiding unnecessary treatment, is required.

In a laboratory that routinely analyzes large numbers of patient samples and requires rapid turnaround of results, evaluating platelets with aggregation could be missed. The simultaneous measurement of platelet counts using the PLT-O and PLT-E methods gives a more specific recognition of platelets. The PLT-O and PLT-E methods should also be rerun in patients with thrombocytopenia, even if there is no aggregation flag from a hematology analyzer.

Examining blood samples using the Mindray MC-80 digital morphology analyzer system on samples that show platelet clumps has the potential to improve PLT-I results in day-to-day laboratory routine.

Authors' contributions

YU contributed to concept and design. KH, EGK drafted work or revised it critically for important intellectual content. All authors have acquired, analyzed or interpreted data. All authors accept responsibility for entire content of this manuscript and approve submission.

Conflict of interest

The authors declare no conflict of interest.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments and uniform requirements for manuscripts submitted to biomedical journals.

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