Hematopathology / Platelet Counting Accuracy in DIC and Acute Leukemia

Accuracy of Platelet Counting by Automated Hematologic Analyzers in Acute Leukemia and Disseminated Intravascular Coagulation

Potential Effects of Platelet Activation

Seon Young Kim, MD,1 Ji-Eun Kim, MS,1,2 Hyun Kyung Kim, MD, PhD,1,2 Kyou-Sup Han, MD, PhD,1 and Cheng Hock Toh, MD, PhD3

Key Words: Platelet count; Acute leukemia; Disseminated intravascular coagulation; Platelet activation

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Abstract

Platelet counting in patients with acute leukemia or disseminated intravascular coagulation (DIC) may have a risk for erroneous counts owing to the presence of nonplatelet particles or platelet activation. We evaluated automated platelet counting methods using the Abbott Cell-Dyn Sapphire (Abbott Diagnostics, Santa Clara, CA), Sysmex XE-2100 (Sysmex, Kobe, Japan), ADVIA 2120 (Siemens Diagnostics, Tarrytown, NY), and Beckman Coulter LH 750 (Beckman Coulter, Miami, FL) compared with the international reference method (IRM). Automated platelet counting methods were inaccurate compared with the IRM, without evidence of interfering nonplatelet particles. It is interesting that platelet activation markers were associated with DIC severity and erroneous platelet counting, suggesting that platelet activation is a potential source of inaccuracy. Furthermore, the artifactual in vitro platelet activation induced a high degree of intermethod variation in platelet counts. The inaccuracy of automated platelet counts increased the risk for misdiagnosis of DIC. More attention needs to be given to the accuracy of platelet counts, especially in clinical conditions with florid platelet activation.

Measurement of platelet counts using automated hematology analyzers is usually quite precise and accurate. However, the accuracy of automated platelet counts can be compromised when measuring very low platelet counts or in the presence of interference from non-platelet particles or platelet abnormalities.1 Recent studies, mainly focusing on the counts of low levels of platelets, demonstrated that automated counts were not as accurate in severely thrombocytopenic samples.2-5 These findings are of concern because current clinical guidelines lowered the prophylactic platelet transfusion threshold to 10 × 10^9/L for patients without additional risk factors.6

In addition to this limitation of the technology, automated platelet counts can be inaccurate even at normal or high platelet ranges owing to the characteristics of blood specimens, eg, in specimens with a substantial amount of interfering particles, including WBC fragments, RBC fragments, immune complexes, bacteria, lipid droplets, or protein aggregates. WBC fragments can cause the spurious elevation of platelet counts in patients with acute leukemia at diagnosis and during chemotherapy.7-11 In 1 study, platelet-like fragments of leukemic blasts were observed in 25% of acute leukemia cases.11 In addition, granulocyte fragments or microorganisms are possible sources of platelet count overestimation in septic patients.12-14 RBC fragments, which are often observed in patients with malignancies as part of microangiopathic processes, are also recognized as a cause of erroneous platelet counts.1

To improve the accuracy of platelet counting and the feasibility of comparing platelet counts between analyzers, the International Council for Standardization in Haematology (ICSH) and the International Society of Laboratory Hematology (ISLH) proposed flow cytometry analysis of monoclonal antibody–labeled platelets and the calculation of...
platelet counts from the platelet/RBC ratio as a new international reference method (IRM). After the establishment of the IRM, multicenter studies comparing counting methods in platelet concentrates were performed. These studies identified considerable variation between the different counting principles and between different instruments using the same counting principle; this variability was suggested to be related to the increased proportion of activated small platelets.

When platelets are activated, they become spherical with a hypogranular cytoplasm and release small particles. This may lead to the erroneous detection of platelets when using automated hematology analyzers owing to their deformed morphology. Patients with acute leukemia or disseminated intravascular coagulation (DIC) have an increased risk not only for interference from nonplatelet particles but also for counting errors due to platelet activation because platelet activation is inevitable during the course of a disease in which high levels of thrombin are generated and many inflammatory cytokines induce platelet activation. Recognizing erroneous results of automated platelet counts in these situations is especially critical for a consistent decision in the diagnosis of DIC and for clinical decision making regarding transfusion. The platelet count is an indispensable parameter in the DIC scoring system proposed by the International Society on Thrombosis and Haemostasis Subcommittee of the Scientific and Standardization Committee on DIC, in which platelet counts of less than $100 \times 10^3/\mu\text{L}$, less than $50 \times 10^3/\mu\text{L}$, and less than $50 \times 10^3/\mu\text{L}$ would score 1 and 2 points, respectively.

The aim of this study was to investigate the accuracy of platelet counting in patients with acute leukemia or suspected of having DIC, clinical conditions with a risk for erroneous platelet counts owing to nonplatelet particles or platelet activation. First, we compared 7 different automated platelet counting methods from 4 widely used hematology analyzers, Abbott Cell-Dyn Sapphire (Abbott Diagnostics, Santa Clara, CA), Sysmex XE-2100 (Sysmex, Kobe, Japan), ADVIA 2120 (Siemens Diagnostics, Tarrytown, NY), and Beckman Coulter LH 750 (Beckman Coulter, Miami, FL), with the IRM. Second, to identify the factors responsible for inaccuracies in automated platelet counting, the interference of nonplatelet particles was examined in some specimens. Third, we analyzed the association of intermethod platelet count variation with platelet activation status. Fourth, we explored whether artificial in vitro platelet activation may induce intermethod variation of platelet counts. Finally, we assessed the impact of the inaccuracy in automated platelet counts on the diagnosis of overt DIC.

Materials and Methods

Study Population

EDTA-anticoagulated whole blood samples submitted to the clinical laboratory for CBC counts were analyzed for 2 cohorts of patients. The “acute leukemia” group consisted of 44 samples from 21 patients with acute leukemia who showed increased peripheral blasts (>20% of WBCs). Besides 21 samples obtained at the initial examination of each patient, 23 samples were repeatedly collected from 10 patients immediately after the initiation of cytotoxic chemotherapy. These samples were included because previous reports suggested an increased incidence of leukemic fragments after chemotherapy. The “suspected-DIC” group consisted of 159 samples from the same number of patients who were clinically suspected of having DIC and had undergone a battery of DIC screening tests. The DIC scores were calculated according to the International Society on Thrombosis and Haemostasis scoring system (scores calculated from platelet count, prothrombin time, fibrinogen, and D-dimer), and “overt DIC” was defined as a score of 5 or more.

Whole blood specimens from 15 healthy volunteers (25-40 years old; platelet count, $125-344 \times 10^3/\mu\text{L}$) were collected with written informed consent for healthy control samples and an in vitro activation study. This study was reviewed and approved by the Seoul National University College of Medicine Institutional Review Board (Seoul, Republic of Korea).

Automated Hematology Analyzers and the IRM

Seven different counting methods from 4 automated hematology analyzers were used as follows: immunologic, optical, and impedance methods using the Abbott Cell-Dyn Sapphire (ImmPLT, CD-o, and CD-i, respectively); impedance and optical methods using the Sysmex XE-2100 (XE-i and XE-o, respectively); optical methods using the ADVIA 2120 (ADVIA); and the impedance method using a Beckman Coulter LH 750 (LH750). The ImmPLT method using the Cell-Dyn Sapphire is a fully automated method using a fluorescein isothiocyanate (FITC)-conjugated CD61 monoclonal antibody, and it generates direct reports of absolute counts. Each analyzer was calibrated according to the manufacturer’s guidelines, and quality controls were performed according to the standard procedures.

The IRM was performed according to the recommended protocol of the ICSH/ISLH. Two monoclonal antibodies,
anti–CD61-FITC (Becton Dickinson, San Jose, CA) and anti–CD41-FITC (Becton Dickinson) were used. Flow cytometry analysis was performed using a FACSCalibur with CellQuest software (Becton Dickinson). RBC/platelet ratios were calculated as described elsewhere,15,20 with the known RBC count determined as the average of each RBC number of small platelets by the total platelet count in the same time platelet counts were measured. MFI of SSC in the gated CD41+/CD61+ platelet events was set out from the scattergram of flow cytometry in the IRM.21 MPC, the mean refractive index of platelets, was obtained using the ADVIA 2120.22 These parameters reflect the internal complexity and density of platelets and decrease when platelets degranulate on activation.21 The pPLT-s was calculated by dividing the number of small platelets by the total platelet count in the ImmPLT method.17 The small platelets, which are defined by CD61+ platelets below a fixed lower threshold in 7θ light scatter, include platelet microparticle fractions and increase after platelet activation.17,23

**Effect of In Vitro Platelet Activation on Intermethod Variation**

The 15 EDTA-anticoagulated whole blood specimens from 10 healthy volunteers and 5 patients (platelet counts, 60-120 10^9/L [60-120 × 10^9/L]; DIC scores, 2-4) were divided into aliquots in 3 tubes. Two aliquots from each specimen were incubated with 0.64 mmol/L fibrin inhibitor (glycine-proline-arginine-proline, Sigma Aldrich, St Louis, MO) for 5 minutes and were then stimulated with 3 or 10 μmol/L adenosine diphosphate (Sigma Aldrich) for 5 minutes at room temperature. The remaining aliquot was kept without manipulation. The platelet counts were obtained using the 7 automated methods and the IRM as described. The degree of platelet activation was estimated by flow cytometric analysis using anti–annexin V–PE and anti–PAC-1–FITC (Becton Dickinson), as well as by the platelet activation parameters obtained from the automated analyzers.
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**Statistical Analysis**

Data were compared by using the Mann-Whitney *U* test and Kruskal-Wallis analysis of variance for continuous variables and the χ² test for categorical variables. The comparison between the 2 methods was performed by using Passing-Bablok regression analysis, the Pearson correlation coefficient, and Bland-Altman analysis. Because the difference between the analyzers and the IRM increased as the platelet counts increased, percentage difference plots were used as suggested by Bland and Altman.24 The 95% limits of agreement (95% LA) were calculated from the 95% range (mean ± 1.96 SD) of the percentage differences to quantify the degree of agreement between the 2 methods.

The percentage of erroneous results between each automated method and the IRM was arbitrarily defined by the percentage of specimens that were outside the ± 25% error limits (± 25% difference from zero difference) of the platelet count. This error limit was modified from the Clinical Laboratory Improvement Amendments criteria for the acceptable performance of the quantitative hematology test in proficiency testing programs.25

The CV across the 7 automated methods and the IRM (intermethod CV) were also calculated for each specimen. The percentage of erroneous results across the quartiles of each platelet parameter were analyzed with adjustments for age, sex, and platelet levels by using linear regression, and the test for linear trend of the percentage was performed on the basis of logistic regression.

Statistical analyses were carried out using MedCalc, version 9.6 (MedCalc Software, Mariakerke, Belgium) and SAS, version 9.1 (SAS Institute, Cary, NC). *P* values less than .05 were considered statistically significant.

**Results**

**Comparison of Platelet Counts Between Automated Methods and the IRM**

The Pearson correlation coefficients between each automated method and the IRM ranged from 0.943 to 0.976 (Figure 1 (upper panels)). The correlation was the strongest between ImmPLT and the IRM and weakest between CD-o and the IRM. Despite these good correlations, the slopes from Passing-Bablok regression were less than 0.90 (from 0.82 in XE-o to 0.89 in CD-i), except for ADVIA (0.95) (Figure 1, upper panels), suggesting that there were considerable proportional errors in platelet counts in the

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**Figure 1** Comparison of platelet counts between the Cell-Dyn Sapphire immunologic method (ImmPLT) (A), optical method (CD-o) (B), and impedance method (CD-i) (C); XE-2100 impedance method (XE-i) (D) and optical method (XE-o) (E); ADVIA 2120 (ADVIA) (F) and Coulter LH 750 (LH750) (G) with the international reference method (IRM) in all samples using Passing-Bablok regression (upper panels) and Bland-Altman analysis (lower panels). Shaded areas in the Bland-Altman plots indicate ± 25% error limits (± 25% difference from zero difference) of platelet counts. Upper panels: A, *y* = 0.867x – 1.187; *r* = 0.976. B, *y* = 0.844x – 1.901; *r* = 0.943. C, *y* = 0.890x – 2.892; *r* = 0.971. D, *y* = 0.859x – 1.587; *r* = 0.969. E, *y* = 0.821x – 2.754; *r* = 0.970. F, *y* = 0.947x – 2.474; *r* = 0.968. G, *y* = 0.844x – 1.144; *r* = 0.971. Outside ± 25% error limits, lower panels: A, 19.7% (40/203). B, 39.9% (81/203). C, 14.7% (28/191). D, 22.7% (46/203). E, 29.0% (58/200). F, 13.4% (27/201). G, 28.0% (56/200). For the CD-i, 12 platelet counts were missing because the CD-i did not report platelet counts on samples with a platelet count of <20 × 10⁹/L. Results were not given for 4 XE-o, 2 ADVIA, and 3 LH750 samples owing to insufficient volume. Platelet counts are given in Système International units; to convert to conventional units (× 10³/μL), divide by 1.0. IRM, international reference method. (Figure 1 continues on next 2 pages.)
automated methods compared with the count in the IRM, except for ADVIA. The 95% LA on the Bland-Altman plots of the ImmPLT, CD-o, CD-i, XE-i, XE-o, ADVIA, and LH750 vs the IRM varied from −46% to +11%, −93% to +37%, −40% to +26%, −54% to +27%, −54% to +24%, −39% to +36%, and −61% to 26%, respectively (Figure 1, solid lines, lower panels). The 95% LA was the widest in the CD-o and narrowest in the ImmPLT. Erroneous platelet count, defined by the percentage of specimens that were outside the ±25% error limits in the Bland-Altman plot, ranged from 13.4% (ADVIA) to 39.9% (CD-o) (Figure 1, shaded area, lower panels).

Investigation of Interfering Nonplatelet Particles

The examination of peripheral blood films revealed no definite evidence of interfering nonplatelet particles in any specimen. Flow cytometric analysis using anti-CD45 in 106 specimens from the acute leukemia (n = 31) and suspected-DIC (n = 75) groups showed no WBC fragments in the platelet-sized area on the scattergram of the IRM. Analysis using anti-CD33 and anti-CD54 antibodies in 14 samples from the patients with acute myeloid leukemia showed no blast fragments. There were no platelet-sized fragments of endothelial cells labeled with anti-CD62E in 33 samples from the definite-DIC group. Flow cytometric analysis with glycoprophorin A in 22 selected specimens also did not show any platelet-sized fragments of RBCs.

Intermethod Variation of Platelet Counts in Terms of Underlying Clinical Condition

The intermethod CV (mean ± SD) of platelet counts was significantly higher in the acute leukemia group (20.7% ± 12.2%) than in healthy control subjects (7.6% ± 1.7%; P < .001) [Figure 2A]. Within the suspected-DIC group, the intermethod CV gradually increased as the DIC severity increased (unlikely DIC, 10.6% ± 4.5%; borderline DIC, 13.4% ± 7.4%; definite DIC, 17.4% ± 13.3% [Figure 2B, Figure 2C, and Figure 2D]. When the acute leukemia specimens were divided into tertiles on the basis of their platelet counts, the 95% LA tended to increase as the platelet levels decreased across all automated methods, and the 95% LA of CD-o was the widest among the automated methods (Figure 2A). Among the suspected-DIC groups, the definite-DIC group showed the widest 95%
LA in all methods, and the unlikely-DIC group showed the narrowest 95% LA (Figures 2B-2D). The ImmPLT demonstrated the narrowest 95% LA across all methods in the 3 subgroups of suspected DIC. In the definite-DIC group, the CD-o, XE-i, and LH750 showed the widest 95% LA (Figure 2B). In the unlikely-DIC group, the 95% LA did not show much variability across all methods and platelet tertiles (Figure 2D).

**Figure 2** Bland-Altman plots showing the mean percentage differences and 95% limits of agreement of platelet counts in different patient groups: acute leukemia (A), definite disseminated intravascular coagulation (DIC) (B), borderline DIC (C), and unlikely DIC (D). The first line represents percentage differences of total specimens in each patient group, and the other lines represent those of tertile subgroups based on platelet levels. Results for 15 healthy subjects are also illustrated in the acute leukemia group (A).
Association of Platelet Activation Markers With Erroneous Platelet Counts and the Underlying Clinical Conditions

To investigate whether platelet activation status had an effect on the erroneous measurement of platelet counts, the percentage of specimens with erroneous platelet counts (outside the ±25% error limits on Bland-Altman plots in Figure 1) was shown on the basis of the quartiles of platelet activation parameters in all specimens (Figure 3).

Intermethod coefficients of variation (ranges) are as follows: A, 20.7% ± 12.2% (7.3%-61.6%); P < .0001; B, 17.4% ± 13.3% (4.3%-64.0%); P < .0001; C, 13.4% ± 7.4% (4.4%-35.1%); P < .0002; D, 10.6% ± 4.5% (5.3%-24.4%); P < .003. P by Mann-Whitney U test compared with the intermethod coefficient of variation of healthy control subjects (7.6% ± 1.7%; range, 4.6%-10.9%). ADVIA, ADVIA 2120; CD-i, Cell-Dyn Sapphire impedance method; CD-o, Cell-Dyn Sapphire optical method; ImmPLT, Cell-Dyn Sapphire immunologic method; IRM, international reference method; LH750, Coulter LH 750; XE-i, XE-2100 impedance method; XE-o, XE-2100 optical method.
Specimens in the lowest quartile (Q1) of the MFI of SSC and MPC, which represent hypogranular activated platelets, showed significantly higher erroneous results than those in the highest quartile (Q4) in the CD-o, XE-i, XE-o, and ADVIA (Figures 3A and 3B). The CD-i and LH750 demonstrated no apparent trends with these parameters. The pPLT-s, the proportion of small and CD61+ platelets representing activated small platelets, showed significant trends of high error rates in the highest quartile (Q4) of pPLT-s for most analyzers (Figure 3C). However, the platelet distribution width showed no apparent increasing or decreasing trends across the quartiles, indicating that platelet size variation was not the main factor responsible for the erroneous platelet count (Figure 3D).

To investigate the activation status of platelets according to the underlying clinical condition, the mean value of the platelet activation parameter in each patient group was calculated as shown in Table 2. Patients with acute leukemia showed significantly lower levels of the MFI of SSC and MPC and significantly higher levels of pPLT-s than patients with unlikely DIC. The definite-DIC group showed significantly lower levels of MPC.

Effects of In Vitro Platelet Activation on the Agreement of Platelet Counts

Fresh whole blood specimens were obtained from 10 healthy volunteers and 5 patients with suspected DIC and were stimulated with a low and a high dose of adenosine diphosphate. Platelet activation status was confirmed with measurements of the percentage of annexin V+ or PAC-1+ platelets, MPC, and pPLT-s (Figure 4). As expected, the intermethod CV was significantly increased after weak and strong activation (control, 7.2% ± 1.4%; weak activation, 7.7% ± 1.4%; and strong activation, 9.9% ± 5.8%, Figures 4A-4C). Similarly, the 95% LA gradually became wider as the intensity of platelet activation became stronger.

![Figure 3](image)

**Figure 3** Adjusted percentage of specimens outside the ±25% error limits in platelet counts by each automated method are presented on the basis of the quartiles (Q1-Q4) of platelet parameters: mean fluorescence intensity of side scatter (MFI of SSC) (A), mean platelet component concentration (MPC) (B), the proportion of small platelets (pPLT-s) (C), and platelet distribution width (PDW) (D).
Impacts of Intermethod Variation in Platelet Counts on the Diagnosis of Overt DIC

We investigated the clinical impact of the intermethod variation in platelet counts on the diagnosis of overt DIC in the borderline-DIC group (n = 64). Platelet counts measured by each automated method were applied to the DIC scoring system and compared with those from the IRM. There was a tendency for all types of analyzers to overdiagnose overt DIC compared with the IRM (false-positive, 3.2%-1.4%; false-negative, 0%-1.6%) [Table 3]. The CD-i, XE-i, and ADVIA showed slightly higher $\kappa$ indices ($\geq$0.90) than the other methods.

Discussion

This study demonstrated that automated platelet counting methods were inaccurate in patients with acute leukemia or DIC when compared with the IRM and that the intermethod variation of the platelet count correlated with the platelet activation status. Furthermore, in vitro platelet activation induced a higher intermethod variation of platelet counts. The inaccuracies of automated platelet counts have an impact on clinical decision making in the diagnosis of overt DIC.

There has been some debate over which counting principle, between the impedance and optical methods, measures platelet counts more accurately. Some studies suggested that the accuracy of the optical methods was superior for thrombocytopenic specimens, while recent studies demonstrated the impedance method to be more accurate for samples from patients undergoing cytotoxic chemotherapy.5,28 Our data showed that there was no consistent superiority of platelet measurement in terms of the counting principle. The ImmPLT and ADVIA gave better agreement with the IRM than the other methods, while the CD-o demonstrated substantial inaccuracy in patients with acute leukemia or severe coagulopathy.

![Graph A](image1)

![Graph B](image2)

All data were adjusted for age, sex, and platelet levels. * $P < .05$, † $P < .01$, and ‡ $P < .001$ compared with the highest quartile (Q4). ADVIA, ADVIA 2120; CD-i, Cell-Dyn Sapphire impedance method; CD-o, Cell-Dyn Sapphire optical method; ImmPLT, Cell-Dyn Sapphire immunologic method; LH750, Coulter LH 750; XE-i, XE-2100 impedance method; XE-o, XE-2100 optical method.
### Table 2

Levels of Platelet Parameters According to Underlying Clinical Condition in 203 Tested Specimens

<table>
<thead>
<tr>
<th>Clinical Condition</th>
<th>Acute Leukemia (n = 44)</th>
<th>Definite DIC (n = 41)</th>
<th>Borderline DIC (n = 64)</th>
<th>Unlikely DIC (n = 54)</th>
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<tr>
<td>MFI of SSC</td>
<td>59.24 (8.47)†</td>
<td>70.84 (14.57)</td>
<td>74.71 (12.39)</td>
<td>73.70 (12.57)</td>
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<tr>
<td>pPLT-s (%)</td>
<td>2.57 (2.39)‡</td>
<td>1.71 (2.12)</td>
<td>2.06 (1.05)†</td>
<td>2.14 (2.21)</td>
</tr>
<tr>
<td>MPC (g/dL)</td>
<td>20.75 (1.08)§</td>
<td>20.60 (1.05)</td>
<td>21.42 (1.21)</td>
<td>21.64 (1.42)</td>
</tr>
<tr>
<td>PDW (%)</td>
<td>15.14 (2.12)</td>
<td>15.88 (2.56)</td>
<td>15.89 (2.23)</td>
<td>15.32 (1.64)</td>
</tr>
</tbody>
</table>

MFI, mean fluorescence intensity; MPC, mean platelet component; PDW, platelet distribution width; pPLT-s, percentage of small platelets; SSC, side scatter.

* Data are shown as the mean (SD). *P* values were calculated by using the Mann-Whitney *U* test and show comparison with the “Unlikely DIC” group.

† *P* < .001.
‡ *P* < .05.
§ *P* < .01.

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**Figure 4** Bland-Altman plots showing the mean percentage differences and 95% limits of agreement of platelet counts in 3 states of platelet activation. Fresh whole blood specimens (n = 15) were stimulated without (control, A) or with 3 μmol/L adenosine diphosphate (ADP) (B) or 10 μmol/L ADP (C). Intermethod coefficients of variation (ranges) are as follows: A, 7.2% ± 1.4% (4.9%-10.4%); B, 7.7% ± 1.4% (4.6%-9.6%); *P* < .164; C, 9.9% ± 5.8% (4.6%-28.9%); *P* < .068. *P* by the paired *t* test compared with control.

A, Annexin V+ (%), 3.9 ± 0.5; PAC-1+ (%), 2.8 ± 0.8; MPC (g/dL), 24.3 ± 1.1; pPLT-s (%), 0.672 ± 0.502. B, Annexin V+ (%), 12.1 ± 4.2 (*P* < .001); PAC-1+ (%), 5.3 ± 1.7 (*P* = .005); MPC (g/dL), 23.8 ± 1.0 (*P* = .083); pPLT-s (%), 0.748 ± 0.577 (*P* = .048). C, Annexin V+ (%), 14.9 ± 1.6 (*P* < .001); PAC-1+ (%), 7.8 ± 4.9 (*P* = .048); MPC (g/dL), 23.8 ± 1.1 (*P* = .027); pPLT-s (%), 0.786 ± 0.581 (*P* = .001). ADVIA, ADVIA 2120; CD-i, Cell-Dyn Sapphire impedance method; CD-o, Cell-Dyn Sapphire optical method; ImmPLT, Cell-Dyn Sapphire immunologic method; IRM, international reference method; LH750, Coulter LH 750; MPC, mean platelet component; pPLT-s, percentage of small platelets; XE-i, XE-2100 impedance method; XE-o, XE-2100 optical method.
The activated platelets are a minor component of the total platelet count; however, when their proportion is significantly increased in pathologic status, it needs to be considered as a factor causing abnormal platelet counts. Although we could not explore the exact mechanism of the association of platelet activation and the intermethod variation, we could postulate that automated analyzers with different counting principles have different intrinsic detection limits for identifying degranulated small platelets.

We finally assessed whether the intermethod variation in automated platelet counts can cause misdiagnosis of overt DIC. There was a potential to overdiagnose overt DIC compared with the IRM when guided by the platelet counts from all types of automated methods because the automated platelet counts tended to underestimate compared with the IRM. One multicenter study using only specimens with a platelet count less than $20 \times 10^3/\mu L (20 \times 10^9/L)$ showed that most automated counting methods had overestimated platelet counts at platelet levels of 5, 10, and 15 $\times 10^3/\mu L (5, 10, and 15 \times 10^9/L)$. However, other studies reported that automated platelet counts were underestimated at the higher levels of 20 or 50 $\times 10^3/\mu L (20 or 50 \times 10^9/L)$, similar to the results described herein. The inaccuracy of automated platelet counts at higher levels could be a matter of serious concern in clinical practice, not only because of the risk for misdiagnosis but also because patients frequently have risk factors for hemorrhage and higher transfusion thresholds.

Despite these inaccuracies of routine automated methods in some abnormal samples, it is not feasible to implement the IRM for routine use as a necessity. Compared with automated analyzers that are much quicker and cost-effective, the IRM is

### Table 3

<table>
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<th>DIC Score</th>
<th>IRM</th>
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<tr>
<td></td>
<td>≥5</td>
<td>&lt;5</td>
<td>FP (%)</td>
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<td>ImmPLT</td>
<td>≥5</td>
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</tr>
<tr>
<td></td>
<td>&lt;5</td>
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<td>34</td>
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<tr>
<td>CD-o</td>
<td>≥5</td>
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</tr>
<tr>
<td></td>
<td>&lt;5</td>
<td>0</td>
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ADVIA, ADVIA 2120; CD-i, Cell-Dyn Sapphire impedance method; CD-o, Cell-Dyn Sapphire optical method; DIC, disseminated intravascular coagulation; FN, false-negative; FP, false-positive; ImmPLT, Cell-Dyn Sapphire immunologic method; IRM, international reference method; LH750, Coulter LH 750; SN, sensitivity; SP, specificity; XE-i, XE-2100 impedance method; XE-o, XE-2100 optical method.

* Patients with a DIC score of ≥5 were diagnosed as having overt DIC.
time-consuming and requires technical expertise and specific facilities. The ImmPLT, another possible option, is not feasible for routine use either owing to its high cost and the requirement of 1 dedicated automated analyzer (Cell-Dyn). Therefore, to improve platelet count accuracy, further efforts to develop a simple and accurate standard method are necessary.

This study has some limitations. First, we performed this accuracy study of platelet counts within 1 clinical laboratory of a tertiary university hospital. Therefore, interinstitutional variation was not investigated. Second, we used routine automated analyzers without advanced calibration of platelet counts just before the study because our study was expected to show intermethod variation only under the current situation of routine automated analyzers that were calibrated on a regular basis according to each of the manufacturer’s guidelines. A universal calibrator would be expected to improve the intermethod variation of platelet counts. Third, although we focused on the readily available platelet activation parameters generated by automated analyzers in this study, these parameters are less sensitive than flow cytometric platelet activation markers such as anti-CD62p and PAC-1. Further analysis using the actual counts of activated platelets measured by flow cytometry would be able to give a more quantitative and specific interpretation.

Although hematology analyzers usually provide reliable platelet counts, they may be inaccurate at enumerating platelets in patients with acute leukemia or DIC, ultimately resulting in the potential risk to make a wrong decision for DIC. It is interesting that platelet activation markers were associated with the severity of DIC and erroneous platelet counts, suggesting that platelet activation is a potential source for the intermethod variation in platelet counts. More attention needs to be given to improve the accuracy of platelet counts, especially in clinical conditions with high levels of platelet activation.

References


