Automated hematology analyzers provide high precision and accuracy for platelet counting.1-3 Platelet (PLT) counting using electroimpedance (PLT-EI), which distinguishes red blood cells (RBCs) from PLTs according to size, is most commonly used. However, giant PLTs or PLT clumping beyond the upper threshold of size may result in underestimation of PLT counts.4 Additionally, spurious increases in PLT counts occur when small particles similar in size to PLTs, such as red cell fragments5 and leukemic cell debris, are present in blood samples.6 Microcytic anemia, which is characterized by small RBCs with mean corpuscular volume (MCV) of less than 80 fL, is another condition that may lead to an overestimation of PLT count when using hematology analyzers. The most common causes of microcytic anemia are iron deficiency, thalassemia, infection, chronic hemorrhagic disorder, and malignant neoplasms.

In this study we compared the PLT counts using electroimpedance (PLT-EI), fluorescent optical (PLT-FO), and manual (PLT-M) methods. We investigated blood samples that contained interfering particles, as identified by inspection of the PLT histogram or the appearance of a flagged result. Blood samples with normal MCV and PLT counts were used as controls. The aims of this study were to identify possible errors in PLT counting when using an automated analyzer, including errors that arise from microcytic RBC interference in blood samples from patients with microcytic anemia, and to identify...

**ABSTRACT**

**Objective:** To determine whether microcytic erythrocytes influence the accuracy of automated platelet (PLT) counting.

**Methods:** We divided a total of 206 K2 ethylenediaminetetraacetic acid (EDTA)–anticoagulated blood samples into 4 groups, as follows: In group 1 (control group), normal mean corpuscular volume (MCV > 80 fL) and PLT count equal or greater than 140,000/μL (n = 45); group 2, normal MCV, reduced PLT count (< 140 x 103 /μL, n = 41); group 3, microcytic samples with normal PLT count (n = 68); and group 4, microcytic samples with reduced PLT count (n = 49). We also compared the platelet counting using electroimpedance (PLT-EI), platelet count using fluorescent optical (PLT-FO), and platelet-count manual (PLT-M) methods, using the Sysmex XE 2100 automatic analyzer.

**Results:** Despite highly significant overall correlations between PLT-EI and PLT-FO, PLT-EI and PLT-M, and PLT-FO and PLT-M ($r = 0.95$ [all $P < .001$]), use of the PLT-EI method resulted in widely overestimated PLT counts in microcytic samples (MCV < 80 fL), compared with use of PLT-FO and PLT-M. Our results identify an MCV of 70 fL as the critical threshold below which PLT-EI became unreliable.

**Conclusion:** The PLT-EI mode overestimated PLT counts compared with PLT-FO and PLT-M modes in microcytic blood. Therefore, PLT-FO is the preferred method for PLT counting in patients with microcytic anemia when using an automated analyzer.

**Keywords:** microcytic anemia, automated hematology analyzer, Sysmex XE-2100, electroimpedance, fluorescent optical, mean corpuscular volume

**DOI:** 10.1309/LM7QPULDM5IHBO3L

**Abbreviations**

$^1$ Signifies equal contributions to the authorship of this article

PLT, platelet; PLT-EI, platelet counting using electroimpedance; RBCs, red blood cells; MCV, mean corpuscular volume; PLT-FO, platelet counting using fluorescent optical; PLT-M, platelet counting using manual; EDTA, ethylenediaminetetraacetic acid; CBC, complete blood count; ANOVA, analysis of variance; CLSI, Clinical and Laboratory Standards Institute

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the critical MCV values that guide the use of different approaches in acquiring reliable PLT counting in patients with microcytic anemic.

Materials and Methods

Grouping of Blood Samples
A total of 206 samples were divided into 4 groups: Group 1 was comprised of specimens with normal (> 80 fL) MCV and normal PLT count (>140,000/μL); this group served as the control group (n = 45). Samples with normal MCV but reduced PLT count were placed into group 2 (n = 41). Samples containing microcytic RBCs and normal PLT counts were assigned to group 3 (n = 68), and those containing microcytic RBCs and reduced PLT counts were assigned to group 4 (n = 49). Our research was carried out in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from all subjects; the institutional review board of Chang Gung Memorial Hospital, Taiwan, approved the study.

Procedures of Blood Sampling
We obtained blood samples via venipuncture and we anticoagulated the samples with K₂ ethylenediaminetetraacetic acid (EDTA). We obtained complete blood count (CBC) measurements within 4 hours using the Sysmex XE-2100 Automated Hematology System analyzer (Sysmex Corporation, Kobe, Japan). We counted all samples containing microcytic RBCs and normal PLT counts were assigned to group 3 (n = 68), and those containing microcytic RBCs and reduced PLT counts were assigned to group 4 (n = 49). Our research was carried out in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from all subjects; the institutional review board of Chang Gung Memorial Hospital, Taiwan, approved the study.

Procedure for PLT-M Counting
We used the PLT-M method according to the instructions provided by the manufacturer of the BD Unopette for Hematology/Chemistry Tests (BD Diagnostics, Franklin Lakes, NJ). Briefly, we transferred 20 μL of a blood sample that had been collected in a UNOPETTE Capillary Pipette (BD Diagnostics), placing the portion of the sample into a UNOPETTE Reservoir (BD Diagnostics) containing 1.98 mL of diluent mixture to achieve a sample to total-volume ratio of 1:100. We then performed PLT counting using a Neubauer hemacytometer (Cat. No.: 0610030, Paul Marienfeld GmbH & Co. KG, Lauda-Königshofen, Germany). Under x400 magnification using the bright-light microscopic technique, we obtained PLT counts for all 25 small squares within the large center square. We then multiplied the count by 1000 to obtain the total PLT count per microliter of blood.

Experimental Protocol and Data Analysis
We acquired PLT counts from samples of all 4 study groups using the PLT-EI, PLT-FO, and PLT-M approaches. We compared the differences in PLT counts obtained via PLT-M and that obtained using PLT-EI and PLT-FO; next, we examined these measurements with respect to the changes in MCV (from <60 fL, 60-70 fL, and 70-80 fL, through >80 fL).

Statistical Analysis
We compared the association among the 3 different methods using a linear correlation test and the Student’s t-test; we used analysis of variance (ANOVA) for comparisons within each group. We performed all statistical analyses using the software program SPSS for Windows, version 11.0 (SPSS Inc., Chicago, IL). Statistical significance was set at P <.05 in all analyses.

Results
We noted the following significant correlations: 0.95; (P <.001) between PLT-EI and PLT-FO (Figure 1, part A), 0.932 (P <.001) between PLT-EI and PLT-M (Figure 1, part B), and 0.97 (P <.001) between PLT-FO and PLT-M (Figure 1, part C). However, compared with PLT-FO and PLT-M, the PLT-EI overestimated PLT counts in microcytic blood samples (MCV < 80 fL) in groups 3 and 4, as indicated by the higher mean (SD) value in those groups (Table 1). In contrast, among the 3 methods, there were negligible differences in PLT counts in samples with MCV of greater than 70 fL (Figure 2, parts A and B). The PLT counts we obtained using the PLT-M method were closer to those acquired with PLT-FO than those with PLT-EI (Figure 2, part C). We observed significant differences in mean PLT count between PLT-EI and the other 2 methods in samples with MCV of less than 70 (Figure 2, parts A and B) for which the mean PLT-EI was higher. The close agreement of the PLT-FO and PLT-M counts for all groups (Figure 2, part C) suggests that the PLT-EI counts may be falsely increased in the microcytic samples.
According to the Clinical and Laboratory Standards Institute (CLSI) guidelines, a discrepancy in PLT counting of more than 25% is considered unsuitable for medical diagnostic requirements. Of the 206 samples we examined in this study, we noted significant PLT-EI versus PLT-FO discrepancies in 37 of the samples from group 3 samples (54.4%), with a mean (SD) discrepancy of 31.1 (4.7) (range, 25.2-44.1); we observed 15 samples in group 4 to have significant discrepancy (30.6%), with a mean (SD) discrepancy of 39.8 (16.8) (range, 25.7-78.4). The diagnoses of the patients enrolled in our study are summarized in Table 2.

Discussion

Although the conventionally used PLT-M counting method performed in the chamber of the Neubauer hematocytometer has been recommended by many laboratories as the reference method, it is time consuming, complicated, and operator dependent. The use of labeled PLTs and fluorescence flow cytometry is a newer approach that was recommended in 2001 by the International Council for Standardization in Hematology and the International Society for Laboratory Hematology. However, the high cost of this method limits its routine use in clinical laboratories. Although it is widely accepted among laboratory professionals that automated hematology analyzers offer high precision and accuracy for PLT counting in healthy patients, these instruments discriminate poorly among
different hematological elements of similar sizes that scatter light within the counting ranges established for the instrument.\textsuperscript{11}

We studied the accuracy of the Sysmex XE-2100 system for counting PLTs in blood samples from patients with microcytic anemia. Our results demonstrated that PLT-FO, set in the reticulocyte-counting mode, could be reliably used for this purpose. The simplicity of this approach also makes it amenable to clinical use. In this method, PLT areas stained by thiazole orange because PLT cells containing residual amounts of megakaryocyte-derived RNA. The procedure can be directly performed using whole blood without preparatory steps and may provide a more accurate PLT counting compared with other methods.

The most important finding of our study is the identification of an MCV of 70 fL as the cut-off threshold below which the

### Table 2. Diagnoses of Patients With Microcytic Blood Samples Showing Significant Discrepancies in Platelet Counts Between the PLT-EI and PLT-FO Methods\textsuperscript{a}

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Group 3\textsuperscript{b}</th>
<th>Group 4\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thalassemia</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>Iron-deficiency anemia</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Hemolytic anemia</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Malignant neoplasms during chemotherapy</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Other diagnoses</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>15</td>
</tr>
</tbody>
</table>

\textsuperscript{a}PLT-EI, platelet counting using electroimpedance method; PLT-FO, platelet counting using fluorescent optical method.

\textsuperscript{b}Significant discrepancy in platelet counting defined as greater than 25% according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).\textsuperscript{4}

\textsuperscript{c}Blood samples with MCV <80 fL, PLT > 140 x 10\textsuperscript{3}/μL.

\textsuperscript{d}Blood samples with MCV <80 fL, PLT < 140 x 10\textsuperscript{3}/μL.
choice of PLT counting method may affect the accuracy of the platelet count result (Figure 2, parts A and B). Below this MCV threshold, our data suggest that impedance PLT-EI platelet counting may overestimate the platelet count. The potential consequences of this methodological bias are that the degree of thrombocytopenia may be under-recognized or that thrombocytopenia may be entirely overlooked in some patients.

A previous study\(^\text{12}\) that compared the accuracy of PLT counting between the impedance and optical methods using 3 different automated analyzers concluded that the precision of impedance methods was better than the precision of optical methods. The difference in their findings and ours may highlight the impact of sample selection on the accuracy of PLT counts. The previous study used blood samples from a wide variety of patient sources (ie, pediatric and adult patients who had renal and liver diseases and cancer, as well as those who had received transplantation and those with unspecified diagnoses) without emphasizing the potential interference caused by microcytic RBCs with PLT counting. In contrast, our study focused on patients with microcytic anemia whose PLT counts were significantly affected by RBC fragment interference.

There are 2 limitations to our study. First, because we did not assess the presence of RBC fragments, we could not evaluate any possible interference by fragments within the platelet PLT-counting methods. In our study procedure, when interference was evident in PLT counting using the automated hematology analyzer, we examined the smear to semiquantitatively assess the PLT count and then correlated the result with that obtained via the PLT-FO method. Second, we did not evaluate the effect of microcytosis on the accuracy of PLT counts at extremely low counts (ie, \(\leq 20,000\)).

To our knowledge, ours is the first report to explore the possible cause of spurious PLT counts in patients with microcytic anemia and to identify an MCV threshold that is critical for ensuring a reliable PLT quantification result and minimize PLT counting errors. Comparing the 2 methods available via the automated analyzer (ie, PLT-EI and PLT-FO) with PLT-M, PLT-FO showed higher consistency in PLT counting for patients with microcytic anemia. PLT-FO is an easy and rapid approach that does not require costly and specialized equipment. We propose that this method could be an alternative to conventional methods of PLT counting in patients with microcytic anemia.

Acknowledgments
This study was financially supported by the research grant of Chang Gung Memorial Hospital, Chang Gung University (grant no. CMRPG 871161). The authors report no personal or financial conflicts of interest. We gratefully acknowledge the volunteers who donated their blood for use in this study.

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