New Fluorescent Method (PLT-F) on Sysmex XN2000 Hematology Analyzer Achieved Higher Accuracy in Low Platelet Counting

Margreet Schoorl, Marianne Schoorl, Jeanette Oomes, and Johannes van Pelt, PhD

Department of Clinical Chemistry, Hematology & Immunology, Medical Center Alkmaar, Alkmaar, The Netherlands.

Key Words: Platelet counting; PLT-O; PLT-F; Sysmex XN2000 hematology analyzer; Transfusion platelet threshold

ABSTRACT

Objectives: In thrombocytopenia, high accuracy and precision of low platelet count is essential for appropriate decisions. The recently introduced Sysmex XN2000 analyzer (Sysmex, Kobe, Japan) offers 3 methods for platelet counting: impedance (PLT-I), optical (PLT-O), and a new fluorescence method (PLT-F). The precision of the PLT-F method in blood samples with platelet counts less than 50 ×10^3/µL (50 ×10^9/L) was investigated and compared with the ICSH CD61-ImmunoPLT reference method. For comparison, PLT-I and PLT-O were determined on the Sysmex XN2000 and Sysmex XE2100 analyzer.

Methods: Blood samples with platelet counts less than 50 ×10^3/µL (50 ×10^9/L) (n = 37) were analyzed on the Sysmex XN2000 and XE2100 analyzers. The CD61-ImmunoPLT method was performed on a Beckman Coulter FC-500 flow cytometer (Miami, FL).

Results: At a platelet count of 20 ×10^3/µL (20 ×10^9/L), reproducibility for PLT-I, PLT-O, and PLT-F on the XN2000 demonstrated coefficients of variation of 9.3%, 8.5%, and 3.0%, respectively. Correlation between PLT-O on the XN2000 and XE2100 yielded an r value of more than 0.977. Linear regression analysis between the PLT-F and CD61-ImmunoPLT methods resulted in a PLT-F of 0.71*CD61 – 0.8 (r = 0.988). Linear regression between PLT-F and PLT-O on the XN2000 resulted in a PLT-F of 1.05*PLT-O – 2 (r = 0.975), and using the transfusion threshold of 20 ×10^9/L platelets resulted in a PLT-F of 0.90*PLT-O – 0.4 (r = 0.956).

Conclusions: The new PLT-F method demonstrated excellent results for reproducibility in samples with platelet counts less than 50 ×10^9/L. PLT-F could be helpful in making better decisions for platelet transfusions.

In subjects with thrombocytopenia, high accuracy and high precision of low platelet count is essential for appropriate clinical decisions. A platelet transfusion threshold of 10 ×10^3/µL (10 ×10^9/L) is recommended for prophylactic transfusion in stable patients, whereas a level of less than 20 ×10^3/µL (20 ×10^9/L) is recommended in case of risk factors such as splenomegaly, coagulation factor deficiencies, and rapid decrease in platelet count or severe bleeding. During follow-up, platelet counts between 50 and 100 ×10^3/µL (50-100 ×10^9/L) are used as an indication of the potential need for platelet transfusion.1-3 These delicate differences require an accurate method for determining the platelet count.

For this purpose, the routinely used Sysmex XE2100 analyzer (Sysmex, Kobe, Japan) is equipped with 2 platelet detection methods, the impedance method (PLT-I) and the optical method (PLT-O). In the majority of samples, platelets are accurately counted using PLT-I. The PLT-O count is more accurate for platelet counts below 100 ×10^3/µL (100 ×10^9/L).4,5 However, for samples from patients undergoing chemotherapy, the impedance count will yield more accurate results.6 Deviations are probably caused by the staining of white cell fragments after apoptosis.7

The Sysmex XN2000 automated hematology analyzer (Sysmex) is a recently launched multiparameter cell counter with innovative methods and an additional possibility for body fluid analysis. New functions have been added to enhance precision in blood cell counting by applying new fluorescent dyes, a nucleated RBC correction function for WBC counting in all specimens (WNR-channel), and a newly added measurement mode for specimens with low WBC counts (LW-mode). In the WBC differentiation channel and abnormal cell detection channel, optimization of the reagent reaction, signal processing, and analysis algorithms have improved the performance in cell differentiation as well as...
in the flagging (interpretive program messages). The RBC parameters and impedance platelets are measured using the same method as the previous XE series of instruments. The reticulocyte channel is the same as on the XE series, including the optical platelet count (PLT-O).

The XN2000 analyzer reveals, in addition to PLT-I and PLT-O methods, an additional feature for counting platelets (PLT-F). The PLT-F channel can be selected for testing on any sample or only used as a reflex test if the RBC or platelet size histograms are abnormal or if the platelet count is below a preset limit (determined by the user). The new PLT-F method is based on a Fluorocell fluorescent dye (oxazine), an extended counting volume, and an extended counting time. Compared with the PLT-O method, platelets are more clearly distinguished from other blood cells using the difference in forward scattered light and the fluorescence intensity

Figure 1 Scattergram of the XN2000 hematology analyzer demonstrating the difference in forward scatter light intensity (FSC) and fluorescence intensity (SFL) of immature (IPF) platelets (PLT) and mature platelets (PLT-F). IPF and PLT-F are clearly distinguished from red blood cells (RBC) and white blood cells (WBC).

Materials and Methods

Preparation of Blood Samples

Blood samples were drawn into Vacutainer tubes, anticoagulated with K₂EDTA (Becton Dickinson, Plymouth, England), and analyzed within 4 hours after collection. Samples from patients with PLT-I counts below 50 × 10³/µL (50 × 10⁹/L) were selected from the daily routine batch. Blood samples were excluded if they showed “flags” indicating the potential presence of platelet clumps. Blood smears were reviewed for the presence of erythrocyte (RBC) or leukocyte (WBC) fragments, microcytosis, giant platelets, and platelet clumps.

Hemocytometry

The calibration status of the Sysmex XE2100 and XN2000 analyzers was initially checked by the manufacturer. On both instruments, quality control samples and maintenance procedures were performed daily according to the manufacturer’s instructions. Blood samples were analyzed with the parameter profile “CBC RETI” (for XE2100) and “CBC RETI PLT-F” (for XN2000) to ensure that results of all parameters were available.

Flow Cytometry

The CD61-ImmunoPLT reference method was performed on the FC-5000 flow cytometer (Beckman Coulter, Miami, FL), according to International Council for Standardization in Haematology guidelines for platelet counting. For comparison, PLT-I and PLT-O were analyzed on the Sysmex XN2000 and the routinely used Sysmex XE2100 analyzers.

Results

A total of 37 K₂EDTA anticoagulated blood samples from patients with PLT-O counts below 50 × 10³/µL (50 × 10⁹/L) was evaluated in the study. During microscopic evaluation of the blood smears, RBC or WBC fragments, microcytosis, giant platelets, and platelet clumps were not observed.

Reproducibility on XN2000 was performed using sequential analysis, repeated 10 times, of samples with low platelet counts. Results are listed in Table II.

Correlation studies were performed against the Sysmex XE2100 analyzer. Results of the correlation between PLT-I...
and PLT-O on XN2000 and XE2100 yielded $r = 0.829$ and $r = 0.977$, respectively [Table 2].

**Figure 2** shows the results for PLT-F on Sysmex XN2000 and the flow cytometric CD61-ImmmunoPLT method. Linear regression analysis to determine the correlation between PLT-F and the CD61-ImmunopLT method resulted in PLT-F = 0.71*CD61 − 0.8 ($r = 0.988$).

Results for PLT-F and PLT-O on XN2000 are shown in **Figure 3**. Linear regression analysis to determine the correlation between PLT-F and PLT-O on XN2000 resulted in PLT-F = 1.05*PLT-O − 2 ($r = 0.975$). Using the transfusion threshold of $20 \times 10^3/\mu$L ($20 \times 10^9/L$) platelets, linear regression analysis between PLT-F and PLT-O on XN2000 resulted in PLT-F = 0.90*PLT-O − 0.4 ($r = 0.956$).

Bias analysis between XN2000 and the CD61-ImmunopLT method demonstrated a unidirectional increasing bias for PLT-F and PLT-O for platelet counts more than $50 \times 10^3/\mu$L ($50 \times 10^9/L$) [Figure 4].

### Discussion

Precise determination of the number of platelets in subjects with thrombocytopenia is important because transfusion of platelets is associated with a higher risk of viral infection, bacterial contamination, and alloimmunization as well as increased costs, whereas underuse carries the risk of hemorrhage. So far, however, determination of the number of platelets is hampered by a certain degree of variation and deviation, in particular when obtained using different methods and/or instruments, especially when the low-range counts are considered.

The use of both impedance and optical methods in automated hematology analyzers results in platelet measurement deviations that might be caused by interference from large platelets or cell fragments of a size similar to platelets. These deviations might be eliminated by the new Sysmex XN2000 analyzer, which offers an alternative method for platelet counting based on a new fluorescence method combined with an extended counting volume and a prolonged counting time.

### Table 2

Linear Regression Analysis of PLT-I and PLT-O Between the Sysmex XE2100 and XN2000 Analyzers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$r$</th>
<th>Slope</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLT-I</td>
<td>0.829</td>
<td>0.85</td>
<td>5.0</td>
</tr>
<tr>
<td>PLT-O</td>
<td>0.977</td>
<td>0.91</td>
<td>−2.4</td>
</tr>
</tbody>
</table>

PLT-I, impedance method of platelet counting; PLT-O, optical method of platelet counting.

* Samples had platelet concentrations less than $50 \times 10^3/\mu$L ($50 \times 10^9/L$) ($n = 37$).
counting time. The aim of the present study was to compare the performance of the PLT-F method using the new Sysmex XN2000 analyzer with that of the Sysmex XE2100 impedance- (PLT-I) and optical platelet count (PLT-O) methods.

Our study revealed excellent results for reproducibility of the new PLT-F method in samples with platelet counts below $50 \times 10^3/\mu L$. The results were consistent with recently published data of Briggs et al.\(^\text{15}\) PLT-F and PLT-O counts determined by XN2000 showed good correlation with the CD61-ImmunoPLT method. In the bias study, the differences for the PLT counts higher than $50 \times 10^3/\mu L$ were greater compared with platelet counts lower than $50 \times 10^3/\mu L$ (Figure 4). In addition, the differences were more distinct for PLT-O compared with PLT-F. The unidirectional increasing tendency indicates that the PLT-O and PLT-F counts determined with XN2000 were consistently lower than those determined with the CD61-ImmunoPLT method. We suggest that the bias might be explained by the statistical variation in the number of cells sampled. In addition, the accuracy of automated platelet counts is a function not only of the underlying technology used to detect platelets but also of the computerized algorithms that are designed to evaluate the platelet data. The CD61-ImmunoPLT reference method is a “dual platform” method in which the measured events are converted using the RBC concentration of the hematology analyzer. To determine which method is the best technique for low PLT counting, the use of a “single platform” flow cytometric method using CD61 together with calibration beads in the same tube should be investigated in a next study.

At PLT levels less than $50 \times 10^3/\mu L$, Bland-Altman difference analysis for PLT-F and CD61-ImmunoPLT method clearly demonstrated a much smaller difference compared with PLT-O and CD61-ImmunoPLT method (Figure 4). This could be explained by the smaller variation coefficient for PLT-F in comparison with the PLT-O method in samples with platelet counts less than $50 \times 10^3/\mu L$.

If the transfusion threshold had been set at $20 \times 10^3/\mu L$, then the PLT-F method would not have resulted in unnecessary transfusions. However, using the PLT-O method would have resulted in 4 cases not receiving proper transfusion (Figure 3). At the transfusion threshold of $10 \times 10^3/\mu L$, only slight differences were observed.

In conclusion, PLT-F on XN2000 is a reliable method for low PLT counting. Because the CD61-ImmunoPLT reference method is not routinely available in all laboratories, we conclude that with respect to the transfusion threshold of $20 \times 10^3/\mu L$, PLT-F is the preferred method to make better decisions on platelet transfusions.

Address reprint requests to Ms Schoorl: Dept of Clinical Chemistry, Hematology & Immunology, Medical Center Alkmaar, Wilhelminalaan 12, 1815 JD Alkmaar, The Netherlands; m.g.schoorl@mca.nl
References