Performance Evaluation of Automated Impedance and Optical Fluorescence Platelet Counts Compared With International Reference Method in Patients With Thalassemia

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• ***Context.***—**Spurious platelet counts from automated methods have been reported in patients with abnormal red blood cells. However, there is no specific study regarding performance of platelet counts by automated methods in patients with thalassemia.**

Objective.—**To investigate the performance of automated platelet counts, including impedance (PLT-I) and optical fluorescent (PLT-O and PLT-F) methods, and compare them with the international reference method (IRM) for platelet counting in patients with thalassemia.**

Design.—**Two hundred forty-nine thalassemia specimens from various subtypes were examined. PLT-I, PLT-O, and PLT-F from a Sysmex XN analyzer were evaluated and compared against the IRM. Demographic data, platelet counts, and red blood cell parameters are shown. Comparability between evaluated methods and IRM, as well as test characteristics, is presented. Factors involving inaccurate PLT-I were analyzed.**

Results.—**Specimens with platelet counts ranging from 31×10^3/μL to 932×10^3/μL were included. Most patients were patients with thalassemia major. Correlation between PLT-I and IRM was lower than that of the other methods in overall patients. PLT-O and PLT-F were correlated to IRM when classifying patients according to clinically significant platelet ranges. All automated methods had acceptable sensitivities; however, specificity of PLT-I was low for diagnosis of thrombocytopenia. High RDW-CV (red blood cell distribution width–coefficient of variation) was an independent factor of inaccurate PLT-I measurement.**

Conclusions.—**Among the evaluated methods, PLT-I was the method least correlated to IRM, with PLT-O and PLT-F comparable to IRM in patients with thalassemia. Optical platelet counts and careful blood smear examination are recommended alternative platelet counting methods, depending on the clinical setting.**

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Platelet count is the component of complete blood count that is used to diagnose quantitative defect in platelets. Accuracy of platelet count is essential for determining bleeding risk in patients. Impedance platelet count is widely used in many clinical laboratories for routine patient testing. In this method, electrical resistance that is produced by passing a particle through an aperture is plotted as a volume histogram. A blood cell or particle fitting the platelet size profile of the platelet distribution curve is counted as a platelet. Although the quality of the impedance method has been continuously improved, inaccurate counting still occurs in many conditions. Abnormal red blood cells (RBCs) with low volume, including microcytes, schistocytes, and spherocytes, have been reported to be the cause of spuriously high platelet counts.[1–3]

A modern technique that combines optical light scattering with fluorescent dyes has been introduced to improve platelet count accuracy.[4–7] Given the importance of platelet count accuracy, the principles of automated platelet counting methods, and the spurious count that may be associated with those methods, a comparative investigation regarding platelet count accuracy and reliability would help clinicians select the best method according to the clinical setting. However, no study has evaluated and compared performance among platelet counting methods in specimens from patients with RBC abnormalities, especially in thalassemia syndrome. This study focused on platelet counts relative to thalassemia syndromes, in which patients usually have a wide variety of abnormal RBCs. The objective of this study was to investigate the performance of automated platelet counts, including impedance (PLT-I) and optical fluorescent (PLT-O and PLT-F) platelet counting methods, and to compare them to the international reference method (IRM) for platelet counting in patients with thalassemia. Test characteristics, including sensitivity, specificity, and concordance rate with the reference method of the evaluated

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automated counts at the thrombocytopenia and thrombocytosis thresholds, were also analyzed. In addition, factors relating to inaccurate platelet counts by impedance method, a commonly used assay in clinical laboratories, were identified and evaluated.

MATERIALS AND METHODS

Patients With Thalassemia and Blood Specimens

Two hundred forty-nine patients with thalassemia who visited the outpatient hematology clinic at Siriraj Hospital, Bangkok, Thailand, during the March 2015–August 2015 study period were included. Thalassemia syndromes included thalassemia minor (α-thalassemia heterozygote, β-thalassemia heterozygote, and hemoglobin [Hb] E heterozygote), thalassemia intermedia (Hb H disease, Hb H disease with Constant Spring, AE Bart disease, and EF Bart disease), and thalassemia major (homozygous β-thalassemia and β-thalassemia/Hb E disease). Patients with history of coexisting anemic conditions other than thalassemia were excluded. Peripheral blood specimens of study participants were collected in dipotassium ethylenediaminetetraacetic acid tubes (Vacuette, Greiner Bio-One, Frickenhausen, Germany) and analyzed for platelet count by various methods within 4 hours after phlebotomy.

Automated Platelet Measurements

The fully automated Sysmex XN-3000 (Sysmex Corporation, Kobe, Japan) was the analyzer that was used to measure platelet counts for the evaluated measurement methods in this study. PLT-I, PLT-O, and PLT-F counts were all analyzed and calculated by using the same modular of Sysmex XN-3000. The PLT-I method uses the direct current hydrodynamic focusing principle and RBC detector count to detect the resistance of the particles passing through the orifice. A distribution curve is then generated. The particles with sizes fitting within platelet-size limits are counted as platelets. For optical fluorescent method, polymethine and oxazine fluorescent dyes are used in the PLT-O and PLT-F analysis, respectively. These dyes stain remnant RNA and nucleic acid–rich organelles in cells and particles. After treatment with these dyes, stained particles pass through the semiconductor laser and a scattergram of side-scattered fluorescent intensity and forward-scattered light is generated and analyzed. The platelet population, which has a high fluorescence intensity, is differentiated from other particles and cells that have a low fluorescence intensity—especially mature RBCs.[5–7] PLT-I was run in the basic complete blood count mode, while PLT-O and PLT-F were run in the reticulocyte and "PLT-F" modes, respectively. At least 3 mL of patient blood was mixed well before testing. Machine processing was performed in manual analysis mode for whole blood, according to manufacturer's instructions. Three levels of quality control materials were regularly evaluated, and required maintenance procedures were performed during the study to ensure proper and accurate functioning of the analyzer.

Red Blood Cell Measurements

Platelet measurements and RBC parameters, including Hb, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin (MCH), MCH concentration, RBC distribution width–coefficient of variation (RDW-CV), and nucleated RBC count, were analyzed by routine complete blood count and reticulocyte modes of the Sysmex XN-3000 analyzer. The analyzer was calibrated for RBC parameter measurement according to the manufacturer's instructions before testing. Quality control materials were checked for all RBC parameters.

International Reference Method for Platelet Counting

The reference method used in this study was the IRM for platelet counting, which is based on the immunoplatelet count recommended by the International Council for Standardization in Haematology and the International Society of Laboratory Hematology.[8] Briefly, 5 μL of well-mixed blood, 5 μL of fluorescein isothiocyanate (FITC)-labeled anti-CD41 (clone HIP8, Biolegend, San Diego, California), and 5 μL of FITC-labeled anti-CD61 (clone VI-PL2, Biolegend) were added into the bottom of the staining tube as separate beads. Then, 135 μL of phosphate-buffered saline solution containing 0.1% bovine serum albumin (BSA-PBS) was added into the tube, and the blood, antibodies, and buffered solution were mixed. After incubation at ambient temperature in the dark for 15 minutes, stained blood was transferred to a standard 12×75-mm polystyrene tube and diluted with BSA-PBS to achieve a final dilution factor of 1:1500 at a total volume of 300 μL. Samples were homogeneously mixed and then immediately analyzed on the BD FACSVerse Flow Cytometer (BD Biosciences, San Jose, California). Before flow cytometric analysis, the instrument was set up, and daily performance quality control evaluation according to the manufacturer's guidelines was performed with BD FACSuite CS&T research beads (BD Biosciences). Acquisition rate was set at medium flow rate (60 μL/min) and the threshold rate was less than 3000 events per second. Data of at least 50 000 RBC and platelet events with a minimum of 1000 platelet events were collected. Gating of RBC, platelet, and RBC per platelet coincidence events was analyzed by FlowJo software (Tree Star Inc, Ashland, Oregon). Corrected platelet count was calculated by dividing the RBC count obtained from the Sysmex XN-3000 by the RBC events to platelet events ratio. With this protocol, IRM of 68 normal blood samples was investigated before the study of patients' specimens. The IRM of normal blood was highly correlated to PLT-I, PLT-O, and PLT-F (data not shown).

Statistical Analysis

PASW Statistics version 18.0 (SPSS Inc, Chicago, Illinois) and R Software version 3.1.2 (R Development Core Team, 2015; Vienna, Austria) were used to analyze data. Continuous variables are expressed as mean ± standard deviation or median, minimum and maximum. Categorical variables are presented as frequencies and percentages. Spearman rank correlation (r_s) was used to assess correlation between continuous data. Unpaired *t* test or Mann-Whitney *U* test was used to compare continuous variables between groups. Pearson χ^2 or Fisher exact test was used to compare proportions of categorical variables between groups. Passing-Bablok regressions were used to evaluate agreement between automated platelet counts and IRM. Evaluated methods were considered to be in agreement with the reference method when the confidence interval of the slope and intercept included the values one and zero, respectively. The true-positive (TP), true-negative (TN), false-positive (FP), and false-negative (FN) rates for comparison between the evaluated methods and IRM were calculated at designated cutoff values. Using TP, TN, FP, and FN rates, sensitivity (TP/[TP + FN]), specificity (TN/[TN + FP]), and concordance rate between evaluated method and IRM ([TP + TN]/[TP + FN + TN + FP]) were calculated. Kruskal-Wallis test was used to compare among the 3 evaluated methods, followed by the Tukey post hoc test for pairwise comparison. Univariate analysis was performed and all variables with P value $< .1$ were analyzed in a multiple logistic regression model to identify independent factors associated with inaccurate PLT-I. Two-sided test was used for all comparisons and a P value $< .05$ was regarded as statistically significant.

Ethical Considerations

The protocol for this study was approved by the Siriraj Institutional Review Board (COA No. Si147/2015).

RESULTS

Demographic data, platelet counts, and RBC parameters of 249 patients are shown in Table 1.

Comparison Between Automated Platelet Measurements and IRM

Patients were categorized into 3 analysis groups according to clinical significance of platelet levels, as follows: IRM less than 100×10^3/μL (thrombocytopenia), IRM between 100 ×

Table 1. Demographic Data, Platelet Counts, and Red Blood Cell Parameters of 249 Patients With Thalassemia

	All Cases (n = 249)	Thalassemia Minor[a] (n = 20)	Thalassemia Intermedia[b] (n = 66)	Thalassemia Major[c] (n = 163)	*P* Values
Age, y					<.001
Mean (SD)	37.9 (15)	53.1 (18.2)	42 (15)	34.3 (12.9)	
Median (Min, Max)	35 (16, 89)	53.5 (22, 82)	44 (16, 78)	31 (16, 89)	
Sex: female	161 (64.7%)	18 (90%)	48 (72.7%)	95 (58.3%)	.006
Transfusion dependence[d]	79 (31.7%)	0	2 (3%)	77 (47.2%)	<.001
Splenectomized status	71 (28.5%)	0	10 (15.2%)	61 (37.4%)	<.001
IRM, $\times 10^3/\mu L$					.43
Mean (SD)	284.7 (205.3)	242.3 (85.4)	228.9 (132.6)	312.5 (233.1)	
Median (Min, Max)	212 (31, 932)	240.5 (43, 377)	210.5 (52, 706)	206 (31, 932)	
PLT-I, $\times 10^3/\mu L$					.21
Mean (SD)	396.7 (250.4)	268.2 (100)	364.6 (213.1)	423.3 (272)	
Median (Min, Max)	322 (40, 1320)	274.5 (68, 535)	319 (54, 1143)	328 (40, 1320)	
PLT-O, $\times 10^3/\mu L$					.29
Mean (SD)	278.3 (187.7)	229 (73.6)	227.6 (124.2)	305.1 (212.6)	
Median (Min, Max)	210 (36, 953)	230 (56, 359)	204.5 (56, 696)	210 (36, 953)	
PLT-F, $\times 10^3/\mu L$					.37
Mean (SD)	276.8 (190.4)	234.2 (78)	226 (127.3)	302.6 (215.3)	
Median (Min, Max)	209 (33, 879)	230 (53, 362)	202 (56, 713)	205 (33, 879)	
Hemoglobin, g/dL					<.001
Mean (SD)	7.5 (1.8)	10.2 (1.7)	7.9 (1.5)	7 (1.6)	
Median (Min, Max)	7.4 (2.3, 17.3)	10.3 (7.2, 13.5)	7.9 (3.6, 12.3)	7 (2.3, 17.3)	
Hematocrit, %					<.001
Mean (SD)	26.7 (6.5)	34.9 (5.8)	31.1 (5.9)	24 (4.8)	
Median (Min, Max)	26.2 (8.4, 53.6)	35.4 (22, 46.7)	30.7 (14, 46)	23.5 (8.4, 53.6)	
MCV, fL					.03
Mean (SD)	70.6 (9.7)	73.2 (8.6)	72.5 (10.4)	69.5 (9.4)	
Median (Min, Max)	70.7 (49.6, 94.3)	73.1 (53.7, 84.6)	74.4 (52, 90.3)	68.9 (49.6, 94.3)	
MCH, pg					<.001
Mean (SD)	19.9 (3)	21.5 (2.9)	18.4 (2.4)	20.4 (3)	
Median (Min, Max)	19.4 (14.3, 30.3)	20.6 (15.5, 26.5)	18.4 (14.3, 27.5)	19.8 (14.9, 30.3)	
MCHC, g/dL					<.001
Mean (SD)	28.3 (2.4)	29.4 (2)	25.5 (2.1)	29.3 (1.5)	
Median (Min, Max)	28.8 (22.1, 33.5)	29.2 (23.7, 32.6)	25.4 (22.1, 30.8)	29.4 (24.5, 33.5)	
RDW-CV, %					<.001
Mean (SD)	27.8 (5.7)	18.9 (5.4)	26.2 (4.4)	29.6 (5)	
Median (Min, Max)	28.3 (13, 40.4)	17.7 (13.1, 37.2)	25.7 (13.9, 38.7)	29.9 (13, 40.4)	
NRBC, /100 WBCs					<.001
Mean (SD)	91.1 (226.8)	0.1 (0.1)	5.8 (39.2)	136.7 (268.4)	
Median (Min, Max)	3.1 (0, 2003)	0 (0, 1)	0.2 (0, 319)	9.1 (0, 2003)	

Abbreviations: CS, Constant Spring; IRM, international reference method; Max, maximum; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; Min, minimum; NRBC, nucleated red blood cell count; PLT-I, impedance platelet count; PLT-O and PLT-F, optical fluorescent platelet count; RDW-CV, red blood cell distribution width–coefficient of variation; SD, standard deviation; WBC, white blood cell.

[a] Thalassemia minor includes α-thalassemia heterozygote, β-thalassemia heterozygote, and Hb E heterozygote.
[b] Thalassemia intermedia includes Hb H disease, Hb H disease with CS, AE Bart disease, and EF Bart disease.
[c] Thalassemia major includes homozygous β-thalassemia and β-thalassemia/Hb E diseases.
[d] Transfusion-dependent patients refers to patients who require red blood cell transfusion at fewer than 8-week intervals.

$10^3/\mu L$ and $450 \times 10^3/\mu L$ (normal), and IRM greater than $450 \times 10^3/\mu L$ (thrombocytosis). Passing-Bablok regression analyses and r_s of platelet counts between IRM and evaluated methods (PLT-I, PLT-O, and PLT-F) are shown in Table 2. Passing-Bablok regression lines between the evaluated methods and IRM at different ranges of IRM are given in the Figure.

Test Characteristics of Automated Platelet Measurements

Cutoff values of IRM at less than $100 \times 10^3/\mu L$ (thrombocytopenia threshold) and more than $450 \times 10^3/\mu L$ (thrombocytosis threshold) were used to calculate test characteristics of PLT-I, PLT-O, and PLT-F. Sensitivity, specificity, and concordance rate of each method at each threshold are presented in Table 3.

Determination of Factors Affecting Inaccurate PLT-I Counts

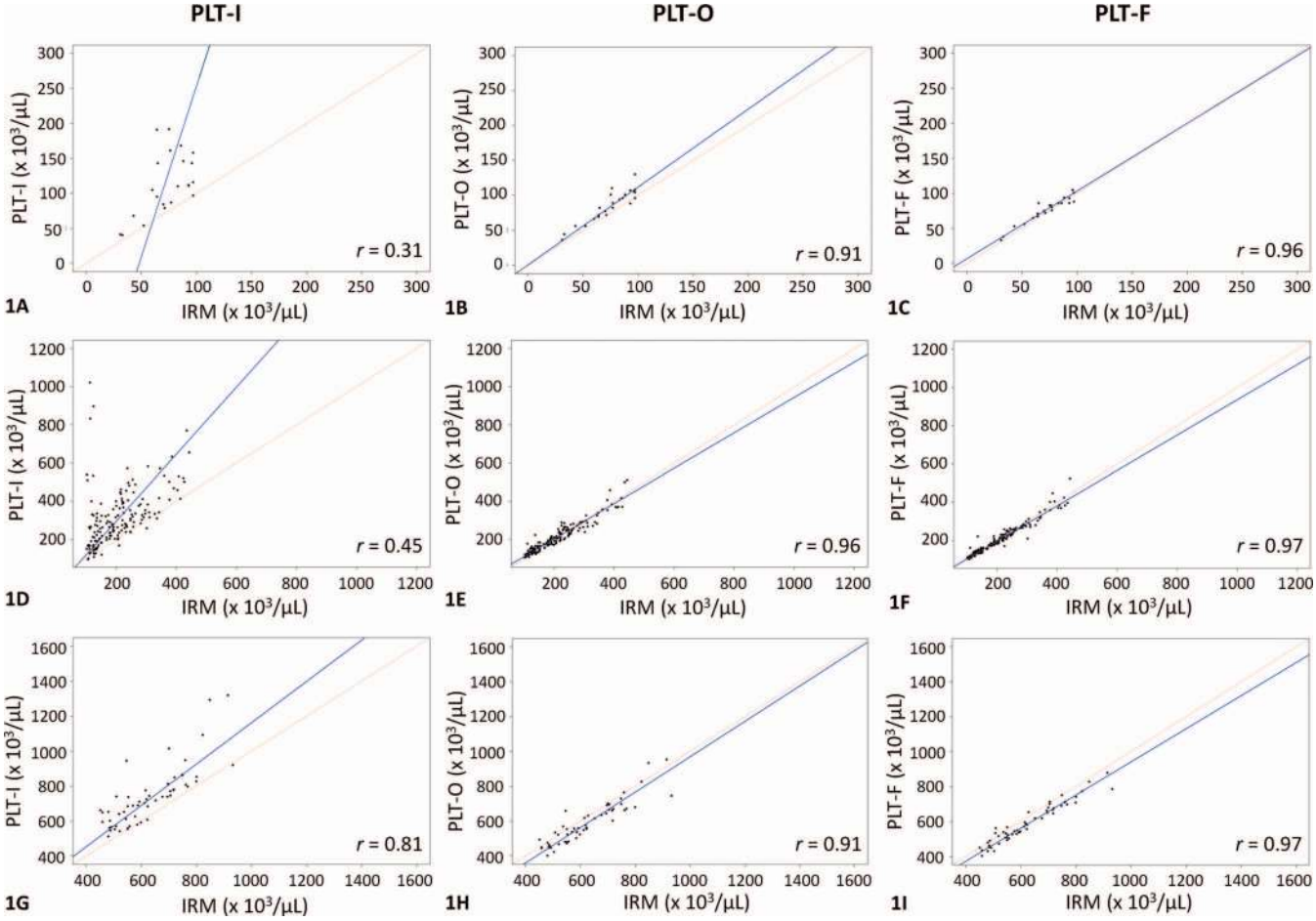
Factors affecting inaccurate PLT-I platelet counts were investigated in this study, because PLT-I is widely available and is often used for routine testing in clinical laboratories. For this study, inaccurate PLT-I was defined as a platelet count by PLT-I method that differed from platelet count by

Table 2. Spearman Rank Correlations and Passing-Bablok Regression Analysis of Platelet Counts Between IRM and Evaluated Methods

Levels of IRM ($\times 10^3/\mu L$)	n	r_s	Slope[a]	Intercept[a] ($\times 10^3/\mu L$)
All cases	**249**			
PLT-I versus IRM		0.82	1.19 (1.13–1.27)	20.64 (4.88–35.85)
PLT-O versus IRM		0.99	0.92 (0.9–0.94)	17.8 (13.61–22.6)
PLT-F versus IRM		0.99	0.92 (0.91–0.94)	11.45 (8.88–13.67)
<100	24			
PLT-I versus IRM		0.31	4.87 (1.8–6.23)	−233.82 (−3296.18 to −24.23)
PLT-O versus IRM		0.91	1.12 (0.99–1.32)	0.35 (−14.8 to 11)
PLT-F versus IRM		0.96	0.97 (0.82–1.05)	6.5 (0.6–16.95)
100–450	170			
PLT-I versus IRM		0.45	1.76 (1.5–2.07)	−56.2 (−114.04 to −14.66)
PLT-O versus IRM		0.96	0.93 (0.88–0.97)	17.56 (8.06–26.21)
PLT-F versus IRM		0.97	0.92 (0.9–0.96)	12.39 (7.68–17.4)
>450	55			
PLT-I versus IRM		0.81	1.18 (0.97–1.52)	−22.18 (−213.54 to 89.62)
PLT-O versus IRM		0.91	1.02 (0.88–1.15)	−47.96 (−131.48 to 37.44)
PLT-F versus IRM		0.97	0.95 (0.88–1.01)	−5.97 (−45.15 to 35.26)

Abbreviations: IRM, international reference method; PLT-I, impedance platelet count; PLT-O and PLT-F, optical fluorescent platelet count; r_s, Spearman rank correlations.

[a] 95% confidence intervals are shown in parentheses.



Passing-Bablok regression lines between evaluated methods (PLT-I, PLT-O, and PLT-F) and IRM: IRM less than 100 × 10³/µL (A through C); IRM between 100 × 10³/µL and 450 × 10³/µL (D through F); and, IRM greater than 450 × 10³/µL (G through I). Solid blue lines, regression lines; dashed pink lines, lines of identity. Abbreviations: IRM, international reference method; PLT-I, impedance platelet count; PLT-O and PLT-F, optical fluorescent platelet count; r, Pearson correlation.

Table 3. Test Characteristics of PLT-I, PLT-O, and PLT-F Compared to IRM at Cutoff Values Less Than $100 \times 10^3/\mu L$ and More Than $450 \times 10^3/\mu L$

Cutoff	Sensitivity[a]	Specificity[a]	Concordance Rate Between Evaluated Method and IRM[a]
$<100 \times 10^3/\mu L$			
PLT-I	0.99 (0.97–1.00)	0.38 (0.21–0.57)	0.93 (0.89–0.96)
PLT-O	1.00 (0.98–1.00)	0.67 (0.47–0.82)	0.97 (0.94–0.98)
PLT-F	1.00 (0.98–1.00)	0.83 (0.64–0.93)	0.98 (0.96–0.99)
$>450 \times 10^3/\mu L$			
PLT-I	1.00 (0.94–1.00)	0.86 (0.80–0.90)	0.89 (0.84–0.92)
PLT-O	0.89 (0.77–0.95)	0.99 (0.96–0.99)	0.96 (0.93–0.98)
PLT-F	0.91 (0.80–0.96)	0.99 (0.97–1.00)	0.98 (0.95–0.99)

Abbreviations: IRM, international reference method; PLT-I, impedance platelet count; PLT-O and PLT-F, optical fluorescent platelet count.
[a] 95% confidence intervals are shown in parentheses.

IRM by more than 25%. This value was derived from the allowable total error of platelet counts, as defined by the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88).[9] Of 249 patient specimens analyzed for this study, 125 (50.2%) PLT-I results were found to be inaccurate. Patient variables, including age, sex, type of thalassemia, RBC transfusion status, splenectomized status, and RBC parameters, were analyzed in univariate analysis for determination of dependent variables of inaccurate PLT-I (Table 4). Variables associated with P value $< .1$, including

Table 4. Univariate Analysis for Determination of Dependent Variables of Inaccurate PLT-I, as Compared to the International Reference Method

Variables	Inaccurate PLT-I (n = 125)	Accurate PLT-I (n = 124)	*P* Values
Age, y			.88
Mean (SD)	37.6 (14)	38.1 (16.1)	
Median (Min, Max)	36 (16, 82)	33 (16, 89)	
Sex: female	82 (65.6%)	79 (63.7%)	.86
Types of thalassemia			.004
Thalassemia minor[a]	3 (2.4%)	17 (13.7%)	
Thalassemia intermedia[b]	37 (29.6%)	29 (23.4%)	
Thalassemia major[c]	85 (68%)	78 (62.9%)	
Transfusion dependence[d]	41 (32.8%)	38 (30.6%)	.82
Splenectomized status	17 (13.6%)	54 (43.5%)	<.001
Hemoglobin, g/dL			.001
Mean (SD)	7.1 (1.5)	7.9 (2.1)	
Median (Min, Max)	7.1 (3.6, 11.8)	7.7 (2.3, 17.3)	
Hematocrit, %			.004
Mean (SD)	25.4 (5.6)	28 (7.1)	
Median (Min, Max)	25.1 (14, 41.9)	26.6 (8.4, 53.6)	
MCV, fL			<.001
Mean (SD)	67.7 (9.6)	73.5 (8.9)	
Median (Min, Max)	67.5 (49.6, 90.3)	74.2 (52.1, 94.3)	
MCH, pg			<.001
Mean (SD)	19 (2.7)	20.9 (3)	
Median (Min, Max)	18.8 (14.3, 26.7)	20.3 (14.4, 30.3)	
MCHC, g/dL			.63
Mean (SD)	28.2 (2.5)	28.4 (2.3)	
Median (Min, Max)	28.7 (22.1, 32.6)	29 (22.1, 33.5)	
RDW-CV, %			<.001
Mean (SD)	29.6 (4.7)	26 (6.1)	
Median (Min, Max)	29.2 (18, 40.4)	26.4 (13, 38.7)	
NRBC, /100 WBCs			.72
Mean (SD)	54.4 (163.1)	128 (272.3)	
Median (Min, Max)	3.4 (0, 1264)	2.7 (0, 2003)	

Abbreviations: CS, Constant Spring; Max, maximum; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; Min, minimum; NRBC, nucleated red blood cell count; PLT-I, impedance platelet count; RDW-CV, red blood cell distribution width–coefficient of variation; SD, standard deviation; WBC, white blood cell.
[a] Thalassemia minor includes α-thalassemia heterozygote, β-thalassemia heterozygote, and Hb E heterozygote.
[b] Thalassemia intermedia includes Hb H disease, Hb H disease with CS, AE Bart disease, and EF Bart disease.
[c] Thalassemia major includes homozygous β-thalassemia and β-thalassemia/Hb E diseases.
[d] Transfusion-dependent patients refers to patients who require red blood cell transfusion at fewer than 8-week intervals.

type of thalassemia, splenectomized status, Hb, hematocrit, mean corpuscular volume, MCH, and RDW-CV, were further analyzed in a multiple logistic regression model. Only high RDW-CV value was found to be an independent factor of inaccurate PLT-I, while splenectomized status was an independent factor of accurate PLT-I.

DISCUSSION

Thalassemia syndromes are important inherited hemolytic diseases in tropical areas and they are now increasing in prevalence worldwide owing to migration.[10] These syndromes are characterized by decrease in globin production and/or hemoglobinopathies in which the spectrum of clinical manifestation varies from asymptomatic to severe hemolytic disease.[11,12] Most patients, however, usually have abnormal RBCs, which may interfere with automated platelet counts. To our knowledge, no study has investigated the performance of automated platelet counts in patients with thalassemia, especially for the automated optical fluorescent methods. Accordingly, the aim of this study was to evaluate the performance of automated platelet counting methods (PLT-I, PLT-O, and PLT-F) compared to IRM counting method in patients with thalassemia. Various types of patients with thalassemia whose IRM counts ranged from thrombocytopenia ($31 \times 10^3/\mu L$) to thrombocytosis ($932 \times 10^3/\mu L$) were included. Most patients were in the thalassemia major group, which consisted of homozygous β-thalassemia and β-thalassemia/Hb E diseases. Values for IRM, PLT-I, PLT-O, and PLT-F were not significantly different among thalassemia minor, thalassemia intermedia, and thalassemia major. Hemoglobin and hematocrit of the thalassemia major group were lower than those of the other 2 thalassemia types. Mean corpuscular volume was not significantly different among the thalassemia types, but MCH and MCH concentration were lowest in the thalassemia intermedia group. Therefore, the abnormalities of RBC parameters did not directly relate to clinical severity. These findings demonstrate the heterogeneity of the syndromes, which may be explained by the variety of genetic abnormalities and treatments (eg, RBC transfusion and splenectomized status).

The authors investigated the performance of platelet counting in overall patients and classified patients according to platelet levels that have or indicate clinical significance. In comparison study, Passing-Bablok analysis revealed nonagreement between the 3 evaluated methods and IRM in patients overall and at the normal platelet level. However, PLT-O and PLT-F were in agreement with IRM at the thrombocytopenic and thrombocytosis levels. Correlations between IRM and PLT-O, and IRM and PLT-F were good for all ranges of platelet counts. Correlation and agreement between PLT-I and IRM were lower than for the other 2 automated methods in patients overall and at every level of platelet count, especially at the thrombocytopenic level. At the thrombocytosis level, all evaluated methods showed agreement and good correlation with IRM. Positive bias trends and higher bias values were associated with PLT-I, when compared to the other 2 evaluated methods. These bias-related findings may be explained by different principles of platelet differentiation among methods. In the Sysmex XN-3000 analyzer, PLT-I and RBCs are analyzed in the same channel and can be separated from each other by their different volume. The volume distribution curve or platelet histogram with lower and upper discriminators produced by the analyzer has an important role for the analysis. The particles with volume between lower and upper thresholds are located under the platelet distribution curve and counted as PLT-I. However, the upper threshold can be flexibly changed owing to the abnormality of each specimen.[1] As a result, abnormally small RBCs, such as microcytes and schistocytes, may be included in the upper region of the histogram and counted as platelets. In addition to abnormal RBCs, RBC membrane–derived vesicles that are generated from oxidative damage to the RBC cytoskeletal membrane in a chronic hemolytic state may play a role in this setting.[13,14] Red blood cell vesicles may have the same size as platelets and may be counted in the same area of the platelet scattergram, leading to spuriously high platelet counts. Previous studies[15,16] have demonstrated the relationship between RBC vesicles and thalassemia syndromes. Those studies showed the number of RBC vesicles to be higher in patients with thalassemia than in the healthy population. This phenomenon was apparent in patients with severe β-thalassemia/Hb E disease and in those who underwent splenectomy—similar to the findings and patients in our study.

Different from the PLT-I method, the PLT-O and PLT-F methods use fluorescent staining of RNA content combined with flow cytometry technique for analysis. Dyes used in the PLT-O and PLT-F systems are polymethine and oxazine, respectively.[17] As a result, the population of platelets can be discriminated from RBCs and RBC vesicles by their higher fluorescent intensity. The authors compared PLT-O and PLT-F in the study group and found that the correlation between methods was excellent (data not shown). Previous studies[18–20] have described good reproducibility and correlation between optical fluorescent platelet counts and IRM, although the populations in these studies did not include patients with thalassemia. Our data confirmed that PLT-O and PLT-F also correlated well with IRM in patients with thalassemia. However, nonagreement between IRM and optical platelet methods was found in patients with normal platelet levels. There is no clear explanation for this phenomenon. The authors hypothesize that an interference factor other than small RBCs may play a role in this nonagreement. Briggs et al[21] reported spurious fluorescent optical counts in patients receiving cytotoxic chemotherapy, which may be explained by the presence of white cell fragments. Platelet activation has been reported as a source of interinstrument variation and inaccurate optical platelet count in disseminated intravascular coagulation and patients with acute leukemia.[22] Specific investigations should be further conducted to demonstrate the effect of non-RBC interference on optical platelet count in patients with thalassemia.

Regarding test characteristics, all 3 automated methods had acceptable sensitivities and concordance with the reference method for diagnosis of thrombocytopenia and thrombocytosis. As such, all 3 automated methods can be used for the screening and ruling out of thrombocytopenia and thrombocytosis in patients with thalassemia. However, the specificity of PLT-I was low for the diagnosis of thrombocytopenia. An additional assay test should be used to improve the specificity of thrombocytopenia diagnosis when the PLT-I value is lower than $100 \times 10^3/\mu L$. PLT-O or PLT-F can be used in this setting owing to their higher specificities and concordance rates with the IRM. However, fluorescent platelet counts cannot be used routinely in every case because of the higher cost, as compared to PLT-I. The

algorithm to switch testing from PLT-I to PLT-O or PLT-F is more appropriate for improvement in diagnostic accuracy. From our data, a high RDW-CV value was an independent factor of inaccurate PLT-I. Accordingly, laboratories should be aware of specimens from patients with this condition. Alternative methods, such as careful blood smear examination combined with fluorescent platelet counting either by PLT-O or PLT-F, are recommended to avoid reporting of inaccurate routine impedance platelet counts. Splenectomized status was found to be an independent factor of accurate PLT-I. The phenomenon is explained by a high platelet count, which is usually found in splenectomized patients. At higher levels of platelet counts, PLT-I tended to be better correlated with IRM (Figure).

The limitation of this study centers on the use of only 1 type of hematology analyzer. Further study in the performance of automated platelet counts in thalassemia and other diseases with abnormal RBCs should be conducted with a variety of hematology analyzers to confirm the performance of the automated platelet counts reported in this study.

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