Comparison of Impedance Platelet Count by Sysmex XE-5000 and Beckman Coulter LH 780 with Optical Fluorescent Platelet Count in Thalassemia Patients

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ABSTRACT

Background: Red blood cell (RBC) abnormalities are frequently found among thalassemia patients and have been reported to be a cause of spuriously high platelet counts.

Objective: To compare impedance platelet count by Sysmex XE-5000 (PLT-XE) and Beckman Coulter LH 780 (PLT-LH) with optical fluorescent platelet count (PLT-O) in thalassemia patients.

Methods: EDTA blood specimens from 152 thalassemia patients were analyzed by routine mode of PLT-XE and PLT-LH. The PLT-O run on reticulocyte mode of Sysmex XE-5000 was used as a reference method. Spearman's rank correlation coefficient (r_s) and Bland-Altman plot were analyzed.

Results: Correlations between impedance and PLT-O methods were excellent, as demonstrated by r_s of 0.95 and 0.97 for PLT-XE and PLT-LH, respectively. However, there was no agreement between impedance and PLT-O methods. There were differences in biases of PLT-XE between thalassemia minor and both thalassemia intermedia and major. Compared to PLT-O, the concordance rates of diagnosis for platelet count abnormality were 94.7% and 96.7% for PLT-XE and PLT-LH, respectively.

Conclusion: Compared to PLT-O, impedance method was associated with spuriously high platelet counts in thalassemia patients. Careful blood smear examination and/or accurate assay should be used in cases suspected of spuriously high platelet count.

Keywords: Hematology analyzer; impedance method; optical method; platelet count; thalassemia (Siriraj Med J 2017;69: 276-282)

INTRODUCTION

Thalassemia syndromes are hereditary hemolytic disorders characterized by decreased globin production and/or hemoglobinopathies. These syndromes are common in tropical areas and are now increasing in prevalence worldwide due to migration.[1,2] Thalassemia syndromes are prevalent in India, Southeast Asia, and the Middle East, and they are considered to be a major public healthcare problem with high disease burden.[3-6] The spectrum of clinical manifestation in thalassemia varies from asymptomatic to severe hemolytic disease, with differences attributed to variations in and degree of genetic abnormalities. Clinically, thalassemia patients can be divided into 3 types: thalassemia minor, thalassemia intermedia, and thalassemia major.[7] Patients with thalassemia minor usually have no symptoms and have normal or near-normal hemoglobin (Hb) concentration. Patients with thalassemia intermedia usually have a Hb level that ranges from 7 g/dL to 10 g/dL.[8] Neither regular transfusion nor splenectomy is required in these patients. In contrast, thalassemia major patients usually have severe anemic symptoms and require regular blood

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transfusion. Splenectomy is performed in some of these patients for the treatment of hypersplenism or to reduce the transfusion requirement.

Although thalassemia syndromes have various clinical manifestations, most patients usually have the same red blood cell (RBC) abnormalities, which are hypochromia and microcytosis.[9] These abnormalities, as well as many poikilocytes (e.g., schistocytes and spherocytes), can be found in moderate to severe forms of thalassemia. These RBC abnormalities have been reported to be a potential cause of spuriously high platelet counts by automated hematology analyzers in many conditions.[10-12] This overestimation of platelets is explained by the interference of platelet counting by abnormally small-sized RBCs in the routine impedance method. Overestimation of platelet counts can lead to the under-recognition of bleeding risk, especially in patients with hypersplenism and thrombocytopenia.

According to our review of the literature, no previous study has specifically investigated automated platelet counts in thalassemia patients. Accordingly, the aim of this study was to investigate the performance of impedance platelet count versus optical fluorescent platelet count (PLT-O) in thalassemia patients. PLT-O was used as a reference method in this study due to its acceptable reproducibility and good correlation with flow cytometry, the international reference method.[13-18] The secondary aim of this study was to evaluate the clinical significance of misdiagnosis of thrombocytopenia when evaluated impedance platelet counts were used.

MATERIALS AND METHODS

Thalassemia patients and blood specimens

One hundred and fifty-two thalassemia patients who visited the outpatient hematology clinic at Siriraj Hospital from January 2013 to June 2013 study period were included in this study. Siriraj Hospital is a 2,000-bed hospital which is located in Bangkok and is Thailand's largest tertiary referral center. Thalassemia patients were divided into the 3 following types: thalassemia minor (beta-thalassemia heterozygote, Hb E heterozygote, and homozygous Hb E), thalassemia intermedia (Hb H disease, Hb H disease with CS, AE Bart's disease, and EF Bart's disease), and thalassemia major (homozygous beta-thalassemia and beta-thalassemia/Hb E disease). Patients with anemia other than thalassemia were excluded. Blood specimens were collected in dipotassium EDTA tubes (Vacuette; Greiner Bio-One, Frickenhausen, Germany) and analyzed for platelet count by the described methods within 4 hours after phlebotomy. Demographic data and values of blood cell analysis were collected and recorded.

Automated analyzers

Patient blood specimens were analyzed by the following 2 automated analyzers: Sysmex XE-5000 (Sysmex Corporation, Kobe, Japan) and Beckman Coulter LH 780 (Beckman Coulter, Inc., Brea, CA, USA). Blood specimens were well-prepared by thorough mixing before the study. Three platelet measurements by two methods were run consecutively. Each blood specimen was evaluated by two impedance methods – once on the Sysmex XE-5000 (PLT-XE) and once on the Beckman Coulter LH 780 (PLT-LH). Then the platelet measurement by optical fluorescent method was consecutively performed on the Sysmex XE-5000 (PLT-O) set in reticulocyte mode. The results of Sysmex XE-5000 reticulocyte mode analysis were used as the RBC reference parameters for this study.

The impedance principle is based on direct current hydrodynamic focusing to detect the resistance of particles passing through the channel. A platelet distribution curve is generated and a platelet count is calculated. The optical fluorescent method (PLT-O) is based on the fluorescent flow cytometry principle and polymethine fluorescent staining of remnant RNA in platelets. In the scattergram, platelets are distinguished from non-platelet particles based on their distinctive fluorescent optical properties. Non-platelet particles, including microcytic RBCs and schistocytes, are characterized by higher fluorescent signals compared to platelets and isolated from platelet population in scattergram.

All methods were performed according to manufacturer's instructions. High, normal, and low levels of QC materials were regularly evaluated. Maintenance procedures were performed during the study to ensure accurate functioning of both analyzers.

Statistical analysis

The data, Bland-Altman plots, and box plots were prepared and analyzed using PASW Statistics version 18.0 (SPSS, Inc., Chicago, IL, USA). Continuous variables were summarized using mean ± SD or median and range (min, max). Categorical variables were presented as number and percentage. Linear regression lines and Spearman's rank correlation coefficient (r_s) were used to assess correlation between continuous data. Bland-Altman plots were used to evaluate agreement between methods by analysis of biases. Bias in this study was defined as a difference between value of evaluated impedance platelet count and PLT-O. The impedance methods were considered to be in agreement with PLT-O when the 95% confidence intervals of mean biases included the value

zero. Categorical data were compared using chi-square test. One way ANOVA or Kruskal-Wallis test was used to compare differences in platelet counts among the three types of thalassemia, followed by post-hoc testing using Games-Howell or Dunn-Bonferroni test. All tests of significance were two tailed and a P value <0.05 was considered to be statistically significant.

Ethical considerations

The protocol for this study was approved by the Siriraj Institutional Review Board (SIRB) (Si 660/2012).

RESULTS

Demographic data, RBC parameters, and platelet counts by the described methods of 152 thalassemia patients are shown in Table 1. Most participants were thalassemia major patients. Hb and hematocrit (Hct) were higher in thalassemia minor patients than in thalassemia intermedia and thalassemia major patients. Degree of decrement in Hb and Hct was correlated with severity of thalassemia. However, RBC indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin; (MCH), and mean corpuscular hemoglobin concentration (MCHC), did not show a marked decrease in thalassemia major like the decrease observed for Hb and Hct. Platelet count by PLT-O method ranged from 66 x 10^9/L to 876 x 10^9/L. For both the impedance (PLT-XE and PLT-LH) and optical (PLT-O) methods, the platelet counts of thalassemia major patients were the highest among all thalassemia groups.

Comparison between evaluated platelet counts and PLT-O

The correlations between the impedance and optical methods were excellent, as demonstrated by the high r_s (0.95 and 0.97 for PLT-XE and PLT-LH, respectively). Bland-Altman plots between evaluated methods (PLT-XE and PLT-LH) and PLT-O are shown in Fig1A and 1B. There was no agreement between the impedance and optical methods, because 95% confidence intervals of mean biases did not include zero.

Differences in platelet counts among the 3 types of thalassemia

Box plots were generated to describe differences in the biases of evaluated methods and PLT-O among the three clinically different types of thalassemia. Statistically significant differences were observed for biases of PLT-XE between thalassemia minor and intermedia groups (P = 0.001) and between thalassemia minor and major groups (P = 0.004). There was no difference in the biases of PLT-XE between thalassemia intermedia and major groups (P = 0.604) (Fig 2A). Multiple comparisons were not performed for the biases of PLT-LH, because significant differences in the biases of PLT-LH were not observed among the 3 types of thalassemia (P = 0.401) (Fig 2B).

Diagnostic discrepancies

Patients were classified into 3 groups based on the clinical significance of platelet levels, as follows: thrombocytopenia for patients with PLT-O less than 100 x 10^9/L; normal platelet count for patients with PLT-O between 100 x 10^9/L and 450 x 10^9/L; and, thrombocytosis for patients with PLT-O more than 450 x 10^9/L. Numbers of thalassemia patients at various ranges of platelet counts when using PLT-XE and PLT-LH compared to PLT-O are shown in Table 2. Compared to PLT-O, the concordance rates for diagnosis for platelet count abnormality were 144/152 (94.7%) and 147/152 (96.7%) for PLT-XE and PLT-LH, respectively. Diagnosis of 5 cases (3.3%) by PLT-XE and 3 cases (2%) by PLT-LH were changed from normal platelet count to thrombocytopenia when PLT-O was used. Diagnosis of 3 cases (2%) by PLT-XE and 2 cases (1.3%) by PLT-LH were changed from thrombocytosis to normal platelet count when PLT-O was used.

DISCUSSION

Spurious or false platelet counts can be reported and these inaccurate results can lead to inappropriate patient management. The interference effect of abnormally small RBCs has been reported as a cause of spurious platelet count in some settings.[10-13] To our knowledge, no study of automated platelet count in thalassemia patients has been published. The present study compared routine impedance platelet counts by two hematology analyzers (PLT-XE and PLT-LH) with optical fluorescent method (PLT-O) in blood specimens from thalassemia patients. Patients with clinical spectrum ranging from thalassemia minor to thalassemia major were included. Platelet counts of patients covered all clinically significant platelet count levels, including mild thrombocytopenia, normal platelet level, and thrombocytosis. Both PLT-XE and PLT-LH had excellent correlations with PLT-O. However, positive biases and disagreement between one or the other impedance method and the optical method were observed. Regarding biases relative to type of thalassemia, the biases for PLT-LH were not statistically significant. However, the bias for PLT-XE in thalassemia minor was lower than for thalassemia intermedia and thalassemia major.

TABLE 1. Demographic data, red blood cell parameters, and platelet counts of 152 thalassemia patients.

	Thalassemia minor* (n=20)	Thalassemia intermedia† (n=28)	Thalassemia major‡ (n=104)
Age (year)			
Mean (SD)	54.6 (18.6)	44.3 (11.8)	34.3 (11.9)
Median (min, max)	52.5 (27, 87)	43 (21, 65)	30 (17, 63)
Gender (female), n (%)	16 (80%)	22 (78.6%)	66 (63.5%)
Hemoglobin (g/dL)			
Mean (SD)	10.2 (1.2)	7.6 (1.5)	7 (1.6)
Median (min, max)	10 (8, 12.1)	7.7 (4.2, 10.8)	7.1 (3.7, 12.9)
Hematocrit (%)			
Mean (SD)	33.2 (3)	29.8 (5.4)	24.1 (4.6)
Median (min, max)	33.1 (27.5, 37.6)	30.4 (16.5, 37.7)	24.4 (13.3, 40.8)
MCV (fL)			
Mean (SD)	70.8 (9.7)	70.1 (9.3)	67.5 (9.5)
Median (min, max)	69.8 (54.6, 89.3)	71.6 (50.6, 84.6)	67.6 (47, 86.6)
MCH (pg)			
Mean (SD)	21.7 (3.3)	17.8 (2.2)	19.5 (2.8)
Median (min, max)	23 (16.3, 28.9)	17.5 (14.4, 24.3)	19.5 (13.7, 26.6)
MCHC (g/dL)			
Mean (SD)	30.6 (1.3)	25.5 (2.1)	29 (2.4)
Median (min, max)	30.6 (29, 33.3)	25.3 (22.3, 29.9)	29.5 (22.7, 34)
RDW-CV (%)			
Mean (SD)	18.1 (1.3)	27.4 (7.1)	28.7 (4.2)
Median (min, max)	16.4 (13.1, 32.6)	25 (17.2, 52.6)	28.8 (15.5, 37.8)
PLT-XE (x 10^9/L)			
Mean (SD)	274.7 (153.1)	313.7 (192.7)	438.6 (264.5)
Median (min, max)	243 (78, 780)	266 (101, 986)	341 (81, 960)
PLT-LH (x 10^9/L)			
Mean (SD)	267.2 (149)	228.7 (137.8)	410.1 (271.8)
Median (min, max)	234 (77, 748)	181.5 (100, 747)	288 (83, 985)
PLT-O (x 10^9/L)			
Mean (SD)	250.2 (152.1)	208.2 (133.5)	371.6 (244.9)
Median (min, max)	232 (75, 788)	177 (75, 789)	254 (66, 876)

* Thalassemia minor includes alpha-thalassemia heterozygote, beta-thalassemia heterozygote, and Hb E heterozygote
† Thalassemia intermedia includes Hb H disease, Hb H disease with CS, AE Bart's disease, and EF Bart's disease
‡ Thalassemia major includes homozygous beta-thalassemia and beta-thalassemia/Hb E diseases

Abbreviations: MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW-CV, red blood cell distribution width - coefficient of variation

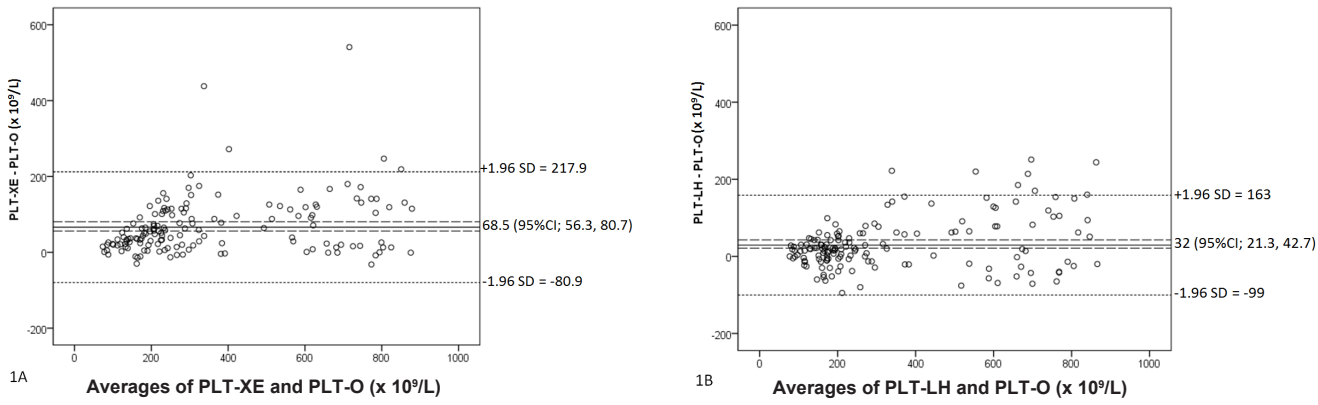


Fig 1. Bland-Altman plots of differences in the evaluated methods and PLT-O: (1A) differences in PLT-XE and PLT-O; and, (1B) differences in PLT-LH and PLT-O. Solid lines indicate mean differences between methods; long dashes represent 95% confidence intervals of biases; and, short dashes denote ±1.96 of standard deviations.

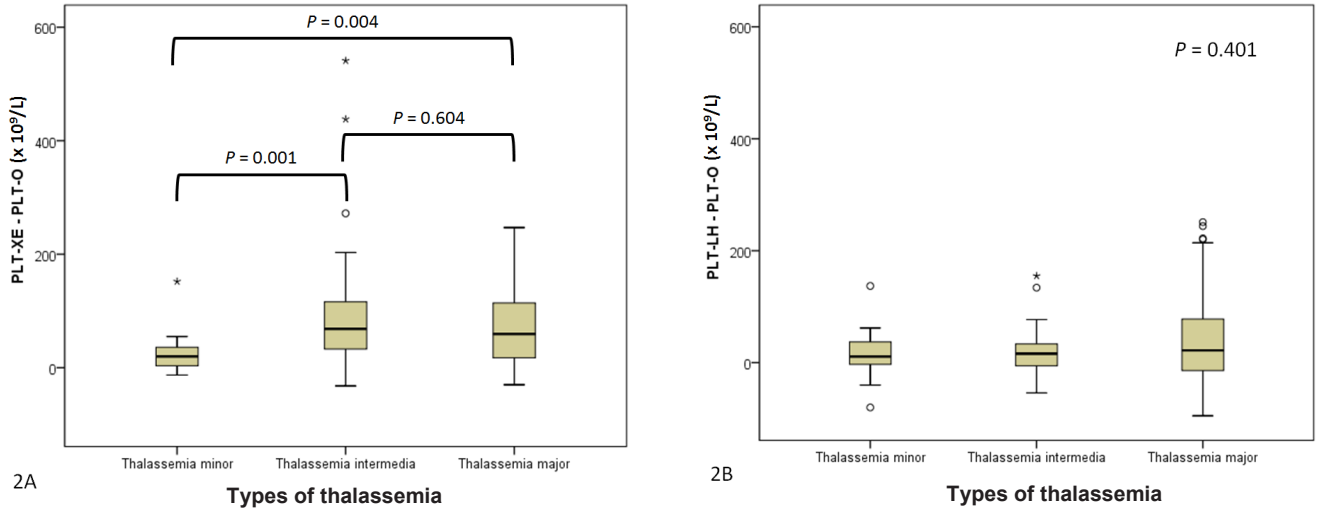


Fig 2. Differences in the biases of evaluated methods among three clinically different types of thalassemia patients: (2A) differences in the biases of PLT-XE; and, (2B) differences in the biases of PLT-LH. Bias in this study was defined as a difference between value of evaluated impedance platelet count and PLT-O.

TABLE 2. Numbers of thalassemia patients at various ranges of platelet counts when using PLT-XE and PLT-LH compared to PLT-O.

	PLT-O (x 10⁹/L)		
	<100	**100-450**	**>450**
PLT-XE (x 10⁹/L)			
<100	5	0	0
100-450	5	96	0
>450	0	3	43
PLT-LH (x 10⁹/L)			
<100	7	0	0
100-450	3	97	0
>450	0	2	43

These findings may be explained by the interference effect of abnormal RBCs, which are usually found in thalassemia patients - even in the mild form. In the impedance method, platelets and RBCs are measured in the same channel. The volume distribution curve or histogram of platelets with lower and upper thresholds is produced by the analyzer for the analysis of platelet count. The particles with volume between these thresholds are added and their sum is reported as the impedance platelet count. The algorithm of analysis is specific to each analyzer. The PLT-XE uses three thresholds to a generate platelet histogram. One threshold is fixed at particle volume of 12 fL, while the other two thresholds are set as the lower and upper thresholds. The upper and lower thresholds of PLT-XE can be at the values 2 fL to 6 fL and 12 fL to 30 fL, respectively. The PLT-LH also sets thresholds and measures the particles with sizes between these thresholds as platelets, although the lower and upper thresholds of PLT-LH are fixed at 2 fL and 20 fL, respectively.[19] As a result, small particles with a size smaller than the determined upper threshold will be falsely counted as platelets by an automated analyzer. These particles include abnormally small RBCs (e.g., microcytes, schistocytes, and spherocytes) which are commonly found in thalassemia patients. Patients with a more severe form of thalassemia (intermedia and major types) tend to have a greater degree of RBC abnormalities. In the present study, positive bias for PLT-XE was slightly higher than that for PLT-LH. The biases for PLT-XE in thalassemia intermedia and major were also higher than that for thalassemia minor. These differences may be explained by differences in the upper thresholds of these analyzers. The upper threshold of PLT-XE covers a particle volume up to 30 fL, while the PLT-LH threshold is set at 20 fL. Therefore, some particles with a size between 20 fL and 30 fL will be included in the platelet population in PLT-XE, but not in PLT-LH.

Regarding the diagnosis of abnormality in platelet quantity, both impedance methods demonstrated a high concordance rate of diagnostic accuracy when compared to PLT-O. Regarding diagnostic discrepancies overall, clinical significance was observed when thrombocytopenic patients were diagnosed as having normal platelet counts by impedance platelet methods. Although the numbers of these patients were small, a failure to identify a patient prone to bleeding could result in inappropriate patient management. Specific flags from the analyzer in this situation should be evaluated and used to alert the clinician to the need for further measurement. Careful blood smear examination and/or accurate assay should be used in cases suspected of having a falsely high platelet count. The algorithm of analysis used and the cost-effectiveness of each method should be studied further.

The weaknesses of this study are the limited number of patients in thalassemia minor and intermedia group and the use of PLT-O as a surrogate reference method. The accuracy of various kinds of hematology analyzers compared to the international reference method of platelet count should be further investigated.

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Authors' Disclosures of Potential Conflicts of Interest

The authors hereby declare no personal or professional conflicts of interest regarding any aspect of this study.

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