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RESEARCH ARTICLE

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Alinity hq platelet count is not impacted by severe microcytosis

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Abstract

Background: Impedance technology has been shown to overestimate platelet (PLT) count in samples with microcytes, while the optical-fluorescence PLT count (PLT-F) by Sysmex has been suggested to be unaffected by microcytosis. The Abbott Alinity hq analyzer employs multi-dimensional optical PLT counting. Our goal was to assess the accuracy of this technology in microcytic samples.

Methods: Platelet measurements were performed by Alinity hq and the impedance (PLT-I) and PLT-F methods on a Sysmex XN-3000 analyzer on 464 samples. PLT concentration range was 6.56–947 $\times 10^9$/L and mean cell volume (MCV) 40.9–123.0 fL. Samples were categorized into normocytic (MCV > 80 fL), microcytic (MCV 65–80 fL), and severely microcytic (MCV < 65 fL) groups.

Results: Alinity hq PLT count showed excellent agreement with PLT-F ($r = 1.00$). Sysmex PLT-I data showed somewhat weaker correlation with both PLT-F and Alinity hq ($r = 0.98$). Increasing bias between Sysmex PLT-I and PLT-F was seen with decreasing MCV values, with mean bias of 35.2 $\times 10^9$/L in severe microcytosis. An inverse relationship was demonstrated between the PLT-I versus PLT-F bias and MCV ($p < 0.0001$). Consistent mean bias was observed between Alinity hq and PLT-F across all MCV ranges.

Mean platelet volume was suppressed or flagged by Sysmex XN in 50% of the samples in the severely microcytic group, and markedly higher red cell distribution width (RDW) was reported compared to Alinity hq (18.1% vs 13.7%, $p < 0.0001$).

Conclusion: The Sysmex PLT-I method overestimated the PLT count in samples with severe microcytosis. Alinity hq provided PLT counts and PLT and RBC indices that were not impacted by microcytosis.

KEYWORDS
impedance, MAPSS™, microcytosis, optical technology, platelet count

1 | INTRODUCTION

Impedance technology has previously been demonstrated to overestimate platelet (PLT) count in samples with microcytosis.[1–5] The most frequent causes of microcytosis are iron deficiency, thalassemia syndromes, and anemia of chronic disease. Tantanate et al[3]. demonstrated that the impedance PLT count by Sysmex XN (PLT-I) showed poor correlation with the International Reference method (IRM), due to high-positive bias and low specificity for the diagnosis of thrombocytopenia, while the optical and fluoresce PLT counts were comparable to the IRM in patients with thalassemia. Several other studies have also concluded that optical or fluorescence-optical

methods are preferred for PLT counting in patients with microcytic anemia, due to the limitations of the impedance PLT technology.[2,4,5]

Impedance counting is the method most commonly used by hematology analyzers. It enumerates and classifies cells based on the momentary change in electrical resistance as they pass through an aperture in a conductive liquid. As the change in impedance is proportional to the particle's volume, this method efficiently differentiates PLT from other cells based on their size; it, however, may produce a spuriously increased count when PLT-sized interferents are present (eg, WBC fragments, schistocytes, spherocytes, microcytes)[1,7] or a spuriously low count, when the sample contains PLT clumps or giant PLTs that fall into the red blood cell (RBC) size range.[1,6,7]

The optical method, used by Abbott instruments and available in reticulocyte counting mode on Sysmex analyzers, discriminates cell types using their light scatter properties, providing better separation between cells of similar size. Optical measurements have shown improved performance compared with impedance in several studies.[8,9] Recently, a comparison of nine analyzers demonstrated that optical measurements of PLT count had consistently better performance for carryover, functional sensitivity, and imprecision compared to impedance.[10]

In addition to the optical technology, the Sysmex XN-series of analyzers also include an optical fluorescent platelet mode (PLT-F) that uses oxazine dye. This method has demonstrated improved accuracy in low PLT ranges.[11,12] In particular, it has been shown to be unaffected by the presence of microcytes.[3,12] The volume of RBC and PLT (MCV and MPV, respectively), however, is still determined by electrical impedance on Sysmex XN analyzers, using size as the only discriminator between them.[13]

The Abbott Alinity hq hematology analyzer employs multidimensional optical technology (advanced Multi-Angle Polarized Scatter Separation, MAPSS™) for PLT enumeration and volume measurements. As cells flow past a laser light beam in a flow cell, they generate eight unique forward, intermediate, and side scatter signals, including a fluorescence signal; six of those are used for separating RBC and PLT and for direct PLT measurements. PLT are differentiated from RBC based on their low intermediate and incident angle light scatters, which are related to the internal composition of the cells. This PLT measurement mode is available as part of the routine complete blood count (CBC).[14,15] The Alinity hq PLT count has recently been shown to be equivalent with the IRM in thrombocytopenia.[15] There is no published information available, however, on the accuracy of Alinity hq PLT count in microcytic samples.

Our goal was to assess the impact of RBC volume, specifically, the presence of microcytic cells on the Alinity hq PLT count and related parameters.

2 | MATERIALS AND METHODS

2.1 | Patients

Four hundred and sixty-four EDTA-anticoagulated peripheral blood samples were selected from the routine workflow of the Clinical Pathology Department of San Giovanni Calibita Fatebenefratelli Hospital, Rome, Italy. All samples were de-identified and used after routine, physician-ordered testing has been completed. An ethics waiver statement was obtained as per the hospital policy for studies using de-identified remnant samples.

The cohort included a large number of anemia cases of various etiologies, such as iron deficiency, anemia of chronic disease, folate or B12-deficiency, thalassemia trait, blood loss, etc. to target a wide MCV range.

2.2 | Automated PLT measurements

Platelet count was generated on each sample within 6 h after phlebotomy as part of a complete blood count with the Alinity hq hematology analyzer (Abbott) and with a Sysmex XN-3000 (Sysmex Corporation), using the PLT-I and PLT-F methods.

Three levels of quality control materials were routinely evaluated on the Alinity hq and Sysmex XN-3000 during the study period.

2.3 | Statistical analysis

Correlation between various PLT methods was assessed by Pearson's correlation. Passing Bablok regression and Bland Altman statistics were used to evaluate agreement between Alinity hq PLT count and Sysmex PLT-I and PLT-F results, with Sysmex PLT-F as reference method. Association between PLT bias and MCV was assessed by Spearman's correlation. Difference between quantitative measurand values between groups was assessed by the Mann-Whitney U test.

Seventeen samples were excluded due to pre-analytical error (inadequate mixing before processing). Additional data were excluded on a case by case basis. If a parameter was marked invalid by an analyzer or method, it was excluded from the analysis.

Statistical calculations were performed using *Analyse-it for Microsoft Excel 5.90*.

3 | RESULTS

Platelet concentration in the cohort ranged from 6.56 to 947 $\times 10^9$/L. MCV values spanned from 40.9 to 123.0 fL (as measured by Alinity hq). Samples were categorized based on their MCV into three groups: normocytic (MCV > 80 fL), microcytic (MCV 65–80 fL), and severely microcytic (MCV < 65 fL).

Alinity hq PLT count demonstrated excellent overall correlation and agreement with the Sysmex PLT-F results (Figure 1A; $r = 1.00$). Sysmex PLT-I results showed good, but somewhat weaker correlation with both the PLT-F method ($r = 0.98$) and Alinity hq ($r = 0.98$; Figure 1B,C).

When bias was assessed on the total cohort, a 7.2 and 9.6 $\times 10^9$/L mean difference was seen between Alinity hq PLT and Sysmex PLT-F and between Sysmex PLT-I and PLT-F results (Table 1). When bias was assessed on the normocytic, microcytic and severely microcytic group separately, Bland Altman analysis revealed increasing mean bias with decreasing MCV values between Sysmex PLT-I and PLT-F, but

a consistent mean bias was seen between Alinity hq PLT and PLT-F across the three MCV ranges (Table 1). The mean bias in the severely microcytic group was 35.2 × 10^9/L between Sysmex PLT-I and PLT-F.

When PLT count bias between Sysmex PLT-I and PLT-F was plotted against MCV, a highly significant inverse relationship was demonstrated ($p < 0.0001$), showing that low MCV (microcytosis) strongly correlates with the overestimation of PLT count by the impedance method (Figure 2). There was no correlation between Alinity hq PLT bias compared to PLT-F and MCV (data are not shown).

Mean platelet volume results are suppressed by Sysmex XN in case of abnormal PLT distribution, which may be caused by uncertain separation between RBC and PLT populations. MPV was suppressed or flagged for review by Sysmex XN in 50% of the samples in the <65 fL MCV group, indicating poor separation between RBC and PLT (Table 2). Interestingly, there was also a significant difference between Alinity hq and Sysmex red cell distribution width (RDW) values in the severely microcytic samples, with Sysmex values being markedly higher compared to Alinity hq (18.1% vs 13.7%, $p < 0.0001$), while good agreement has been obtained on the normocytic group (13.4% vs 13.6%; Table 3).

4 | DISCUSSION

Our findings have demonstrated that the Sysmex PLT-I method has overestimated the PLT count in samples with severe microcytosis compared to Sysmex PLT-F, while Alinity hq PLT counts were equivalent with Sysmex PLT-F results regardless of MCV.

Although several interfering substances and conditions are known to potentially impact PLT measurements, the most important factor that determines the accuracy of the results is the proper separation between RBC and PLT.[1,6] This may be challenging in the presence of small erythrocytes (schistocytes, spherocytes, microcytes) and large platelets or PLT clumps, especially for impedance methodologies.[1–7] Several automated analyzers employ multiple PLT counting technologies in addition to impedance (optical, optical fluorescence, or immunolabeling) to increase measurement accuracy.[7–9,12] These methods often require additional testing and the use of additional reagents. In addition, even when alternative technologies are used for PLT measurements, the volume of the cells is still determined with electrical impedance by most analyzers, and this has potential impact on related parameters that depend on accurate separation of PLT and RBC, such as MPV or RDW. A PLT counting method that is available for every CBC without additional testing and reagents and is not subjected to the same limitations as electrical impedance would provide increased efficiency and improved workflow for clinical laboratories.

Alinity hq PLT results showed high level of correlation and agreement with Sysmex PLT-F results, consistent with earlier findings.[15] The 7.2 × 10^9/L mean bias is likely attributed to calibration and was similar to the bias seen between Sysmex PLT-I and PLT-F data (9.6 × 10^9/L). The bias between Sysmex PLT-I and PLT-F displayed a strong dependency on MCV; specifically, the lower the MCV, the higher was the bias ($p < 0.0001$). This is likely caused by the high number of microcytes in samples with an MCV of <65 fL, causing unclear separation between the RBC and PLT volume histograms. This

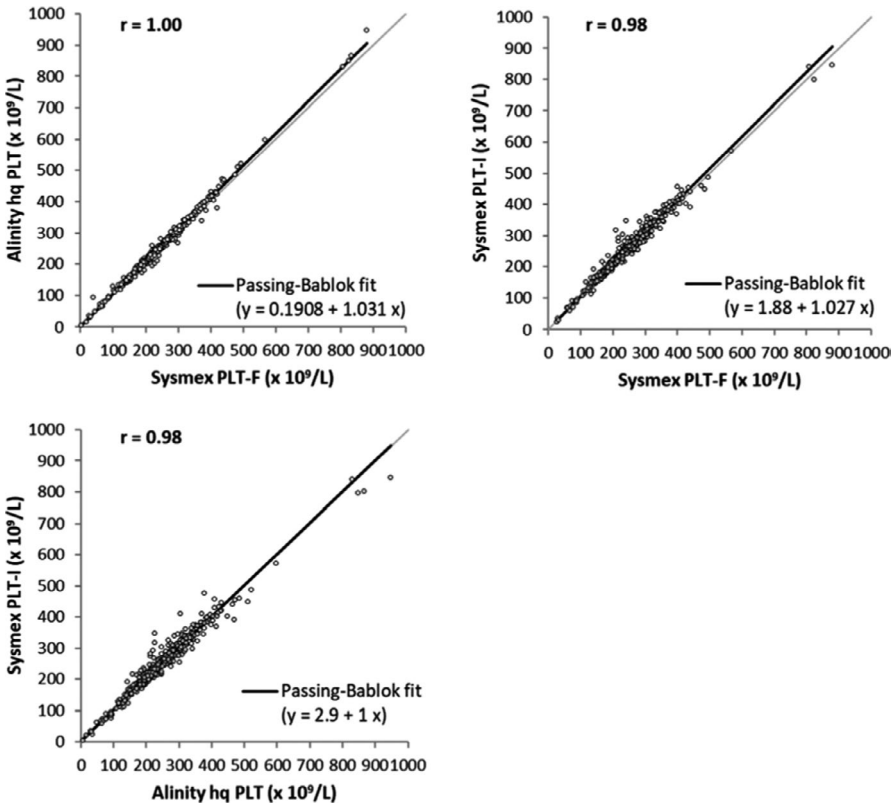

FIGURE 1 Passing Bablok regression between Alinity hq PLT and Sysmex PLT-F (A), Sysmex PLT-I and PLT-F (B), and Alinity hq PLT and Sysmex PLT-I (C). *r*, correlation coefficient

finding aligns with the observation by Pan et al[5], who has concluded that the PLT count produced by electrical impedance becomes unreliable below MCV of 70 fL. The uncertain separation between RBC and PLT is further supported by the finding that the Sysmex analyzer suppressed MPV results in 13 and flagged them for review in additional 5 out of the 36 severely microcytic samples. MCV, however, was still reported for all these samples, as was RDW. The RDW by Sysmex was found to be significantly increased compared to Alinity hq. If unclear separation caused the MPV being suppressed, it is possible that the overlap between the RBC and PLT volume histograms might have incorrectly increased the volume distribution of the RBC histogram, resulting in higher RDW. This hypothesis is further supported by the findings of Tantanate et al[3], who has shown that high RDW-CV was an independent factor for overestimating PLT-I count by Sysmex XN-3000 in a thalassemic population.

TABLE 1 Mean PLT concentration bias between Alinity hq and Sysmex PLT-F and between PLT-I and PLT-F according to MCV

		Mean bias ($\times 10^9$/L; 95% CI)	
MCV	*n*	PLT-I vs PLT-F	Alinity hq vs PLT-F
All (40.9–123 fL)	447	9.6 (8.1–11.2)	7.2 (6.3–8.1)
<65 fL	36	35.2 (25.0–45.3)	9.2 (4.9–13.5)
65–80 fL	79	14.4 (10.0–18.7)	7.1 (4.3–9.8)
>80 fL	332	6.0 (4.8–7.3)	7.0 (6.1–8.0)

Abbreviations: CI, Confidence interval; fL, femtoliter; MCV, Mean corpuscular volume; PLT-F, Fluorescence platelet count; PLT-I, Impedance platelet count.

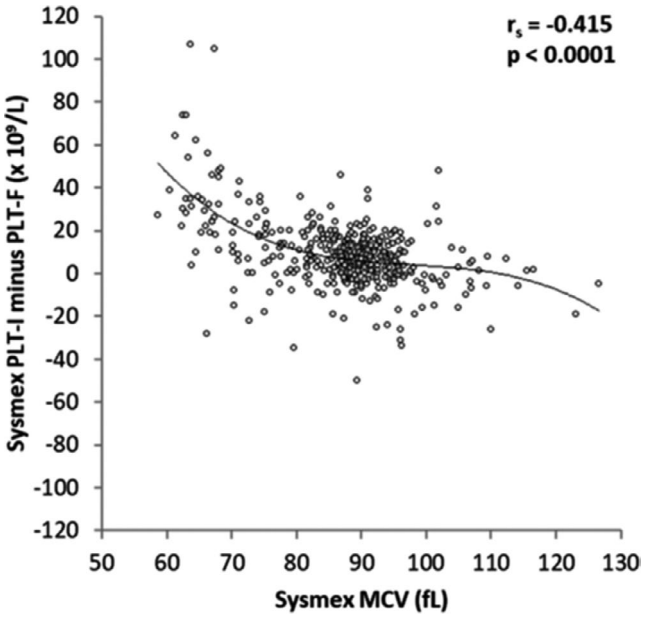

FIGURE 2 Spearman's correlation between PLT count bias (Sysmex PLT-I vs PLT-F) and the MCV of the samples. r_s, Spearman's rank correlation coefficient

The bias between Alinity hq and PLT-F results, on the other hand, was consistent across normocytic, microcytic, and severely microcytic samples. There was no correlation between PLT bias (compared to PLT-F) and MCV. MPV values were reported for all 36 severely microcytic samples, although were flagged for review in 9 of them. Even in the MCV of 65–80 fL subgroup, Alinity hq had only two MPV results flagged for review, while the Sysmex analyzer suppressed MPV in four and flagged it in another eight samples. RDW by Alinity hq was lower in the severely microcytic population compared to Sysmex. We have previously demonstrated that the Alinity hq RDW is a good discriminator between iron deficiency anemia (IDA) and beta thalassemia trait (BTT) due to the RDW being lower in BTT compared to IDA.[16] The Sysmex RDW, however, was unable to differentiate between these two conditions, because BTT patients showed an increased RDW, similarly to IDA.[16] This observation may be related to the potential overestimation of RDW by Sysmex in severely microcytic samples, due to the uncertain separation between RBC and PLT populations.

Platelet count in the thrombocytopenic ranges has important clinical significance for assessing bleeding risk and the need for PLT transfusion. It has recently been suggested that the threshold for prophylactic PLT transfusion could be further reduced to as low as 5.0×10^9/L in patients without risk factors[17]; it was, however, noted that the analytical performance and lack of harmonization of automated PLT counts are posing challenges for this goal.[17] Microcytic anemia is a frequent condition; therefore, additional studies, confirming our findings and raising awareness about methodological limitations would be important for the prevention of reporting inaccurate PLT result and for ensuring proper management of thrombocytopenia in this patient group. Our study has also described a previously unrecognized potential impact of unreliable RBC and PLT separation on MPV and RDW, which may contribute to the

TABLE 2 Number of suppressed (by Sysmex), invalid (by Alinity hq), flagged for review (by Sysmex), or flagged as Suspect (by Alinity hq) MPV results according to MCV

		Suppressed or invalid result		Flagged result	
MCV	*n*	Alinity hq MPV	Sysmex MPV	Alinity hq MPV	Sysmex MPV
<65 fL	36	0	13	9	5
65–80 fL	79	0	4	2	8
>80 fL	332	0	6	6	15

Abbreviations: fL, femtoliter; MCV, Mean corpuscular volume; MPV, Mean platelet volume.

TABLE 3 RDW (median and interquartile ranges) by Alinity hq and Sysmex XN according to MCV

MCV	N	RDW median and interquartile ranges (%)		p (Alinity hq vs Sysmex XN)
		Alinity hq	Sysmex XN	
<65 fL	36	13.7 (131 – 17.2)	18.1 (17.3 – 20.3)	<0.0001
65–80 fL	79	15.5 (14.2 – 18.0)	17.0 (15.2 – 19.1)	0.014
>80 fL	332	13.4 (12.8 – 14.6)	13.6 (12.9 – 14.9)	N.S

Abbreviations: fL, femtoliter; MCV, Mean corpuscular volume; MPV, Mean platelet volume; N.S., Not significant; RDW, Red cell distribution width.

well-published technology- and platform-specific differences for these measurands.

Alinity hq employs only optical and fluorescence flow cytometry principles for generating all measurands. The use of various light scatter signals allows for the differentiation between RBC and PLT based on their internal structure, in addition to size. Therefore, this technology has the potential to provide accurate separation between RBC and PLT. Our results have demonstrated that Alinity hq provides accurate PLT count in samples regardless of MCV and provides PLT and RBC indices that are not impacted by microcytosis.

CONFLICT OF INTEREST

Gabriella Lakos and Zainab Mukhtar were employees of Abbott at the time of the conducting the study and drafting the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on reasonable request from the corresponding author.

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