International Journal of Laboratory Hematology

WILEY

ISLH International Journal of Laboratory Hematology

ORIGINAL ARTICLE OPEN ACCESS

Definition of Significant Platelet Clumping: Should We Review All Samples With a Platelet Clumping Flag From Automated Hematology Analyzer?

Suhyeon Woo[1] | Bohyun Kim[1] | Nam Hun Heo[2] | Min-Sun Kim[1] | Young Ahn Yoon[1] | Young-Jin Choi[1]

[1]Department of Laboratory Medicine, Soonchunhyang University Cheonan Hospital, Soonchunhyang University College of Medicine, Cheonan, Korea | [2]Clinical Trial Center, Soonchunhyang University Cheonan Hospital, Soonchunhyang University College of Medicine, Cheonan, Korea

Correspondence: Bohyun Kim (bhkim@schmc.ac.kr)

Received: 4 April 2024 | **Revised:** 14 September 2024 | **Accepted:** 5 October 2024

Funding: This study was supported by the Soonchunhyang University Research Fund.

Keywords: automated hematology analyzer | flag | platelet clumping | platelet count | pseudothrombocytopenia

ABSTRACT

Objectives: This study aimed to determine a definition for significant platelet clumping (PC) and evaluate the performance of the Sysmex XN instrument for detecting platelet clumps.

Methods: For part 1, 372 specimens with a 'PLT_clump?' flag in XN-9000 were classified into five groups according to the average number of PCs. We compared the initial platelet count (measured by XN-9000 using impedance method) and corrected platelet count (counted optically or re-analyzed by XN-9000 using vortexed or re-collected sample) of each group. For part 2, 1000 specimens with a PC flag divided into three subgroups {group N (PC = 0), Y (PC ≥ 1), and Z (microscopic fibrin clot)} and additional two groups {group S (PC(+) specimens without any flag and with flags of other categories) and group NC (negative control)} were collected. Positive predictive value (PPV), negative predictive value (NPV), sensitivity and specificity of PC detection of XN-9000 were obtained and the platelet counts and four indices (PDW, MPV, P_LCR, and PCT) of groups NC, N, Y, Z, and S were compared to detect PC more precisely.

Results: In part 1, all groups showed significant difference between the initial and corrected platelet counts. In part 2, PPV, NPV, prevalence, sensitivity, and specificity were 41.5%, 56.5%, 43.4%, 2.18%, and 98.3%, respectively. The platelet counts and four indices showed statistical differences for detecting PCs, and especially PDW and P_LCR were significantly smaller in group Z than group N or Y.

Conclusions: We suggest the definition of significant PC by the presence of at least three platelets. In addition, utilizing platelet-related indices should be developed to improve the efficiency of the PC detection.

1 | Introduction

Pseudothrombocytopenia (PTCP) is a phenomenon where a platelet (PLT) count performed by an automated hematology analyzer is falsely low because platelet clumps (PC) result in an abnormal histogram and inaccurate enumeration of platelets [1, 2]. PC can be formed due to pre-analytical and analytical factors [2, 3]. Among these, the effect of ethylenediaminetetraacetic acid (EDTA) which is an anticoagulant added to a sample for a complete blood count (CBC) is a major cause of strikingly large clumps [1, 3, 4]. When platelets are exposed to EDTA, conformational change of membrane protein will occur and previously

hidden epitopes will be revealed. If either acquired or naturally occurring IgM, IgG or IgA autoantibodies against those epitopes are present, platelets are aggregated [2, 4].

PTCP including EDTA-induced PTCP is not strongly associated with a specific disease, nor does the presence of PTCP mean an elevated risk of certain disease. Nevertheless, although its prevalence is low, PTCP can appear simultaneously in various conditions such as autoimmune disease, pregnancy, viral infection, and treatment with some drugs. It can also occur in healthy individuals [2, 4, 5]. In these patients, failure to identify a low platelet count due to PTCP can lead to unnecessary platelet transfusion, bone marrow biopsy, or misdiagnosis of hematologic neoplasm. Such cases have been constantly reported [2], It is reasonable to assume that there are many more undetected cases. In addition, in a rare case, platelet clumps could mask thrombocytosis and the diagnosis of hematologic neoplasms associated with thrombocytosis might be delayed.

Therefore, it is important not to miss out any case of spuriously low platelet count and report the accurate platelet count in clinical laboratory. However, it was only a few years ago when the first written guideline for platelet counting in an automated hematology laboratory from the French-speaking Cellular Hematology Group (GFHC) [1] and a comprehensive review on PTCP were published [2]. In addition, to our best knowledge, there are only two systematic research studies evaluating the flagging performance of the Sysmex XN series (Sysmex, Kobe, Japan), one of the most popular automatic hematology analyzers for PC detection [6, 7]. Although these initial studies have provided valuable insights into PTCP and platelet counting, they have some limitations. First, there was no clear or specific and detailed definition of PC. Although GFHC suggested to define it by the presence of at least five attached platelets, it was based on their consensus only without providing any experimental evidence [1]. Two research studies also used previously suggested or arbitrary definition of PC without any validation [6, 7]. Second, with regard to the PC detecting ability and PC flagging algorithm of the Sysmex XN series one of those systematic research studies did not fully reflect a real laboratory circumstance because it used citrate blood samples which were artificially induced to aggregate by adding adenosine diphosphate (ADP) [6]. In addition, although fibrin clot could generate a PC flag, those studies did not consider it [1, 6, 7].

Thus, this study aimed to understand the significance of PC better in the context of a modern automated hematology laboratory. Our study consists of two parts. In the first part, we tried to statistically determine a definition for PC by collecting real blood samples with PC and analyzing difference between initial PLT count with Sysmex XN-9000 and final corrected PLT count. The second part was for evaluating performance of the Sysmex XN instrument for detecting PC and revealing its little-known algorithmic features in PC flagging.

2 | Materials and Methods

2.1 | Specimens

In our laboratory, we collect the sample for CBC test using BD Vacutainer K2 EDTA 5.4 mg tube. The EDTA blood sample is analyzed by Sysmex XN-9000 within 30 min after sampling. The maximum limit of sample analysis time is 1 h after sampling, and nearly 100% of blood samples are analyzed within the limit. If there are more than one platelet-related flags including the "thrombocytopenia" flag, which are made when the platelet count is $< 20 \times 10^9$/L, the platelet count was measured by PLT-F mode by XN-9000 and peripheral blood smear slide is made by SP-10 automated slidemaker/stainer. Microscopic examination of those slides is performed by skilled laboratory personnels to confirm any abnormalities of platelet.

From March 3 to June 25, 2022, examiner A (a skilled medical technician with 6 years of experience) reviewed all smear slides automatically made by SP-10 and collected all initial CBC results including platelet indices and blood smears made from EDTA-anticoagulated whole blood specimens from inpatient wards, outpatient clinics and emergency room with a "PLT_clump?" flag (PC flag) in the XN-9000 until the number of specimens reached 1000. Samples with incomplete CBC data, samples with a missing smear, and samples collected from neonatal intensive care unit or other special units were excluded.

For part 1, 372 specimens from initial 7 weeks were used. For part 2, 1000 specimens from the total study duration were used. In addition, if any laboratory personnel encountered specimens not flagged by a platelet-related one but did contain PC, these specimens were also collected (group S). Thus, group S contained samples without any flag and samples only with flags of other categories: white blood cell-related flags and red blood cell-related flags. Examiner B (a laboratory medicine doctor with 3 years of experience) reviewed every sample with other flags except "PLT_clumps?" for 4 days to gather specimens with neither platelet-related flag nor PC as a negative control group (NC). In all parts, multiple samples per patient were permitted.

2.2 | Automated Complete Blood Cell Analyzer

We used a Sysmex XN-9000 system (Sysmex, Kobe, Japan). In this system, platelets were counted with an impedance method. Platelet distribution width (PDW), mean platelet volume (MPV), platelet large cell ratio (P_LCR), and plateletcrit (PCT) were automatedly calculated based on the results measured by an impedance method and reported with the platelet count. In our laboratory, fluorescence platelet counting on PLT-F channel was performed in a case where a platelet count was $< 20 \times 10^9$/L. PC flag was generated based on default flagging thresholds (Q-value of 100 measured in the WNR/WDF channel) and information processing unit version 22.12. A blood smear was prepared with a Sysmex SP-10 automated slidemaker/ Wright-Giemsa stainer (Sysmex, Kobe, Japan) which made blood smear slides in the same way as a human technician. The process of Wright-Giemsa staining by SP-10 was as follows: Wright solution: 3 min ➔ Wright solution + buffer: 3 min ➔ Giemsa solution + buffer: 5 min ➔ Washing by distilled water: 1 min ➔ Drying: 10 min. The quality of blood smear slide was verified by skilled laboratory technician and doctor.

2.3 | Part 1: Defining PC

During the collection of specimens, if the second platelet count result and smear were obtained after mixing the originally

clumped specimen by vortex machine (Vortex-Genie 2; Scientific Industries, Bohemia, New York, USA) at 2000 rpm for 30 s [8, 9] or recollecting another sample in a sodium citrate tube, they were also collected. All collected smears were then reviewed again by examiner B and classified using the following criteria by consensus among examiners A and B. At first, microscopic examination was performed at 200× magnification. The presence of PC was defined as more than two platelet clumps (PAs) consisting of at least three platelets on the smear. If there was no PC, the sample was assigned to one of two groups based on the existence of microscopic fibrin clot, an amorphous basophilic material usually located at the end of the smear: N (PA = 0) and Z (presence of microscopic fibrin clot). If there was a PC, the sample was assigned to one of three groups based on the average number of PAs in a field at 1000× magnification: A ($0 < PA < 1$), B ($1 \leq PA < 5$), and C ($PA \geq 5$) (Figure 1). When a sample was categorized as group A–C, collected second smear was reviewed. If PC was completely resolved, the second platelet count produced by XN-9000 was adopted as the final count. An optical count was conducted for the second smear with persistent PC using the Fonio method [10] by the second examiner. This third count was adopted as the final count in these cases. The reliability of platelet counting was assessed beforehand via intraclass correlation coefficient (ICC) analysis of the optically counted number of platelets and the number reported by the XN-9000 for 30 samples without any platelet-related flags: ICC (95% CI) = 0.991 (0.980–0.996).

To determine the definition of PC that was statistically significant, initial and final platelet counts of groups A–C were compared via paired *t*-test. To weigh its clinical significance, medical records were reviewed. IBM SPSS version 26.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Results were considered significant if the p value was < 0.05.

2.4 | Part 2: Performance of PC Detection of Sysmex XN Series

A total of 1000 specimens with PC flag from the total duration were classified into three groups by consensus among two examiners: N (PA = 0), Y (PA > 0) and Z (presence of microscopic fibrin clot). Group S and NC which were mentioned above were also included (Figure 2).

For performance evaluation of the Sysmex XN series, both positive predictive value (PPV) and negative predictive value (NPV) of a "PLT_clump?" flag were calculated and sensitivity and specificity of PC detection were estimated. In addition, we compared the platelet counts and four indices (PDW, MPV, P_LCR, and PCT) of groups NC, N, Y, Z, and S via analysis of variance (ANOVA) with post hoc Tukey's test to reveal their relationships in PC flagging. The same statistical program and p- value threshold was employed.

2.5 | Ethics Statement

This study was approved by the institutional review board of Soonchunhyang university Cheonan hospital (2022–04-013).

3 | Results

3.1 | Part 1: Defining PC

For 7 weeks, 37 554 CBC tests were performed by the Sysmex XN-9000. Among them, 824 specimens were flagged as "PLT_clump?" Among them, 372 CBC results and blood smears were collected. They were classified into five groups via light microscopic

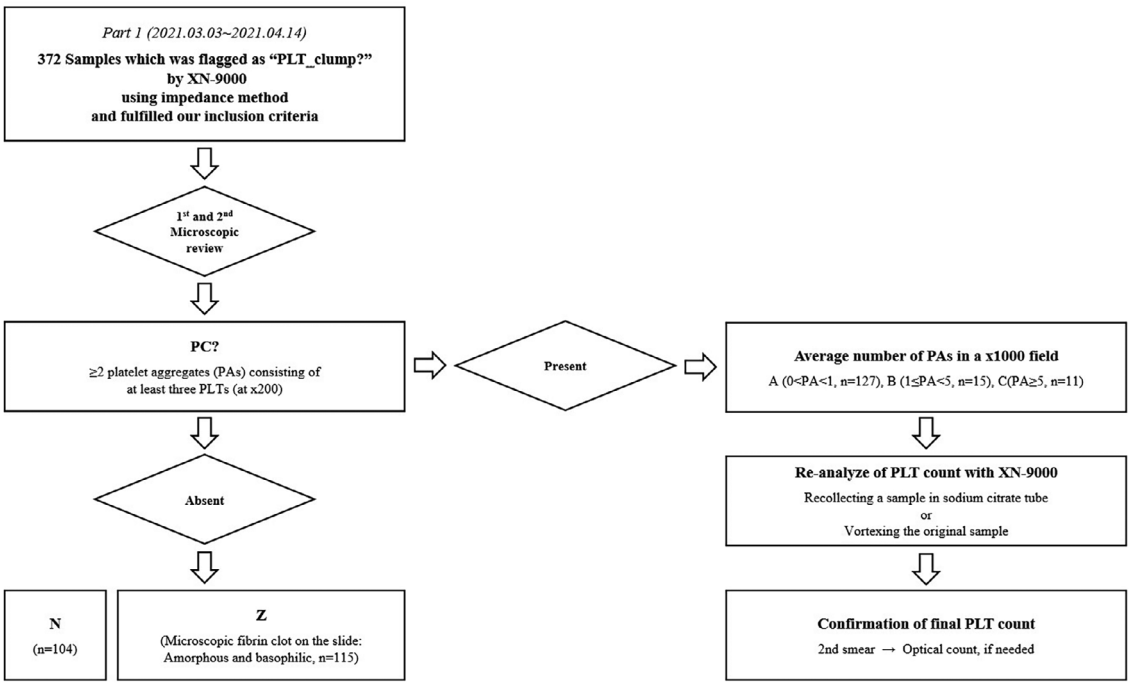

FIGURE 1 | Flowchart of specimen collection and classification for part 1.

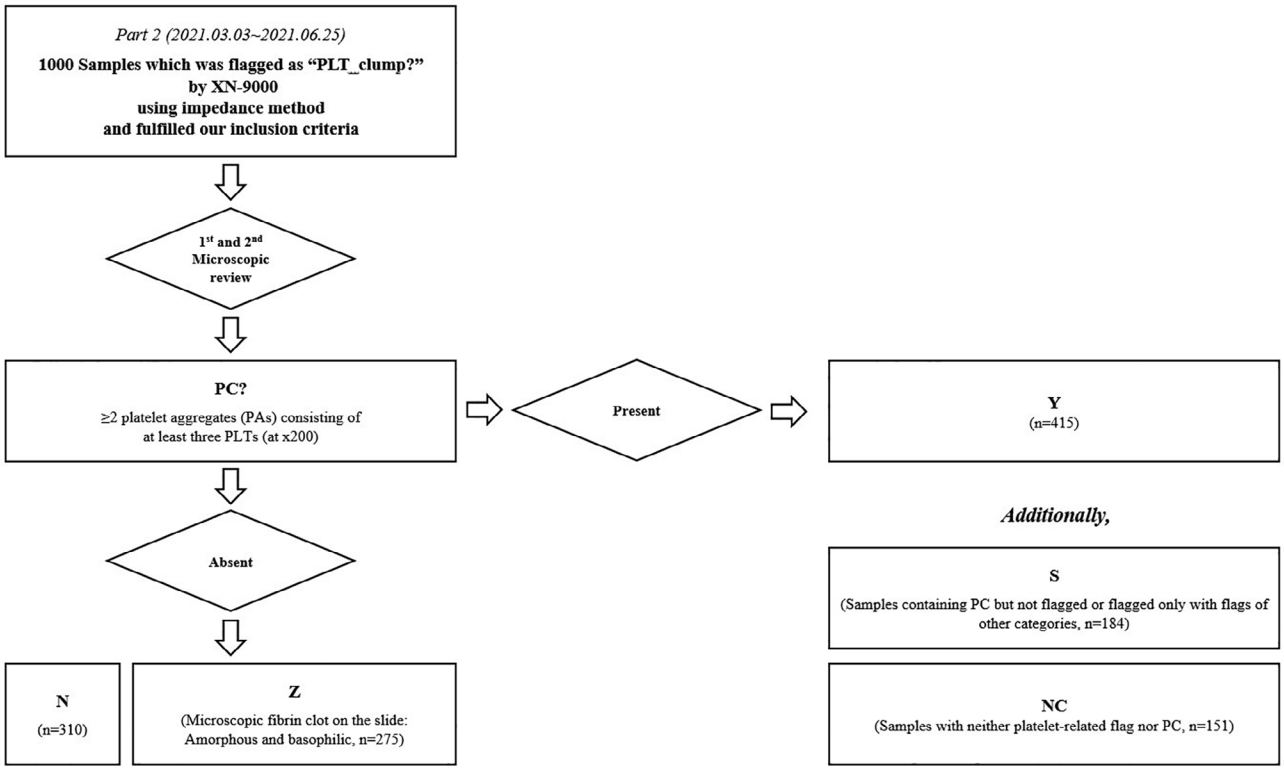

FIGURE 2 | Flowchart of specimen collection and classification for part 2.

TABLE 1 | Differences between initial and final platelet counts of each group. All groups showed significant difference between initial and final platelet counts.

Group[a]	Platelet count ($\times 10^9$/L) {Mean (Standard deviation)}		Paired difference {Mean (95% confidential interval)}	*p*
	Initial	Final (Second or third)		
A ($n=127$)	211.73 (105.98)	294.09 (145.89)	82.36 (70.49–94.30)	<0.001
B ($n=15$)	307.87 (151.50)	459.80 (220.18)	151.93 (10.57–293.29)	0.036
C ($n=11$)	84.55 (58.53)	312.00 (148.80)	227.46 (143.47–311.44)	<0.001

[a]The group was assigned based on the average number of platelet clumps (PAs) consisting of at least three platelets in a field at 1000× magnification: A ($0<PA<1$), B ($1\leq PA<5$), and C ($PA\geq 5$).

review: N ($PA=0$: $n=104$, 27.96%), Z (presence of microscopic fibrin clot: $n=115$, 30.91%), A ($0<PA<1$: $n=127$, 34.14%), B ($1\leq PA<5$: $n=15$, 4.03%), and C ($PA\geq 5$: $n=11$, 2.96%). Among 153 samples of group A–C, 26 samples were re-analyzed after vortexing and 20 samples were re-anlyzed after recollection.

When the initial platelet count reported by the XN-9000 and the final count (i.e., the second platelet count obtained after vortexing or recollecting a sample or the third optical count) of groups A–C were compared, all groups showed significant difference (A: $p<0.001$, B: $p=0.036$, and C: $p<0.001$, Table 1). The mean difference was 82.36×10^9/L for group A and 227.46×10^9/L for group C (Table 1).

Among group A, a total of 37% of specimens showed a clinically important difference in initial and final counts: from thrombocytopenia to a normal count ($n=30$, 23.6%) or from a normal count to thrombocytosis ($n=17$, 13.4%). Thus, there were 30 cases of pseudothrombocytopenia among 372 analyzed specimens.

3.2 | Part 2: Performance of PC Detection of Sysmex XN Series

During the period of specimen collection, a total of 101 810 CBC tests were ordered and 2355 samples were flagged as "PLT_clump?" by the XN-9000. Of them, 1000 CBC results and blood smears were collected and classified into three groups: N ($PA=0$: $n=310$, 31%), Y ($PA>0$: $n=415$, 41.5%) and Z (presence of microscopic fibrin clot: $n=275$, 27.5%). In group S, 184 specimens were included. While examiner B reviewed 368 samples with flags except "PLT_clumps?" for 4 days, 160 specimens actually contained PC, 57 specimens did not contain PC but were flagged with other platelet-related flags, and 151 specimens were included in group NC.

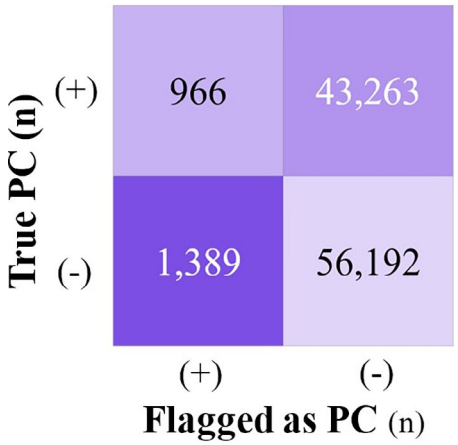

FIGURE 3 | Confusion matrix of "PLT_clump?" (PC) flag; Sensitivity of 2.18%, Specificity of 98.3%, Positive predictive value (PPV) of 41.5%, and Negative predictive value (NPV) of 56.5%.

Therefore, the PPV of a "PLT_clump?" flag reported by the Sysmex XN series was 41.5% (Group Y/Total = 415/1, 000) and the NPV of a "PLT_clump?" flag was 56.5% (Specimens did not contain PC but were flagged with other platelet-related flags + Group NC/Specimens with flags except "PLT_clumps?" = (57 + 151)/368). Meanwhile, the prevalence, sensitivity, and specificity were only able to be estimated approximately because we did not review microscopically for all 101 810 CBC tests. To begin with, the number of true positives was calculated as 977 (Specimens with PC flag × PPV = 2355 × 0.415) and the number of true negatives was calculated as 56 192 (Specimens without PC flag × NPV = 99 455 × 0.565) (Figure 3). Accordingly, the prevalence, sensitivity, and specificity were estimated as 43.4%, 2.18%, and 98.3%, respectively.

Relationships in the platelet number and indices among groups NC, N, Y, Z, and S are shown in Table 2. Regarding the number of platelets and PCT, there were statistical differences in total ($p < 0.001$). There were differences in the order of N < NC < Y, Z < S in details. PDW showed a significant difference in total ($p < 0.001$). There were significant differences between groups in the order of NC, S, Z < N, Y. As for MPV, statistical difference in total was revealed ($p < 0.001$), and differences in the order of S < NC < N, Y, Z were also seen. For P_LCR, there was significant difference in total ($p < 0.001$) and differences between groups in the order of S < NC < Z < N, Y.

To understand differences in platelet number and indices among groups NC, N, Y, Z and S at a glance, we plotted 1335 dots of groups NC, N, Y, Z, and S in three two-dimensional spaces (Figure 4).

4 | Discussion

4.1 | Part 1: Defining PC

In clinical laboratories, a blood smear is usually reviewed microscopically to detect the presence of PC when its platelet count is lower than the laboratory's own criteria or it is flagged as

TABLE 2 | Comparison results of the platelet counts and four indices (PDW, MPV, P_LCR, and PCT) of groups NC, N, Y, Z, and S via analysis of variance (ANOVA) with post hoc Tukey's test to reveal their relationships in PC flagging {Results are expressed as mean (standard deviation)}. There were statistically significant differences in platelet count and all platelet-related indices among 5 groups ($p < 0.001$) For platelet count and PCT, the differences are in the order of N < NC < Y, Z < S. For PDW the differences among 5 groups in the order of NC, S, Z < N, Y. For MPV, the differences in the order of S < NC < N, Y, Z were seen. Finally, for P_LCR, the differences among 5 groups in the order of S < NC < Z < N, Y were seen.

	Total ($n = 1335$)	NC[a] ($n = 151$)	N[a] ($n = 310$)	Y[a] ($n = 415$)	Z[a] ($n = 275$)	S[a] ($n = 184$)	p	Post hoc Tukey's test
PLT count ($\times 10^9$/L)	183.32 (113.09)	160.07 (120.51)	112.83 (80.14)	199.28 (115.28)	196.28 (87.03)	265.77 (112.05)	< 0.001	N < NC < Y, Z < S
PCT	0.19 (0.11)	0.16 (0.11)	0.12 (0.09)	0.22 (0.12)	0.21 (0.09)	0.26 (0.11)	< 0.001	N < NC < Y, Z < S
PDW (fL)	12.17 (2.14)	11.47 (2.21)	12.58 (1.71)	12.83 (2.01)	12.06 (2.18)	10.82 (2.14)	< 0.001	NC, S, Z < N, Y
MPV (fL)	10.71 (0.90)	10.38 (1.00)	10.93 (0.59)	10.97 (0.77)	10.73 (0.91)	10.04 (1.07)	< 0.001	S < NC < N, Y, Z
P_LCR	30.07 (7.06)	27.58 (7.95)	31.94 (4.49)	32.17 (5.93)	29.97 (6.98)	24.60 (8.50)	< 0.001	S < NC < Z < N, Y

Abbreviations: MPV, mean platelet volume; P_LCR, platelet large cell ratio; PCT, plateletcrit; PDW, platelet distribution width; PLT, platelet.
[a]The groups were defined as follows: NC (Samples with neither platelet-related flag nor PC), N (Samples without PC), Y (Samples with PC), Z (Samples with microscopic fibrin clot), and S (Samples containing PC but not flagged or flagged only with flags of other categories).

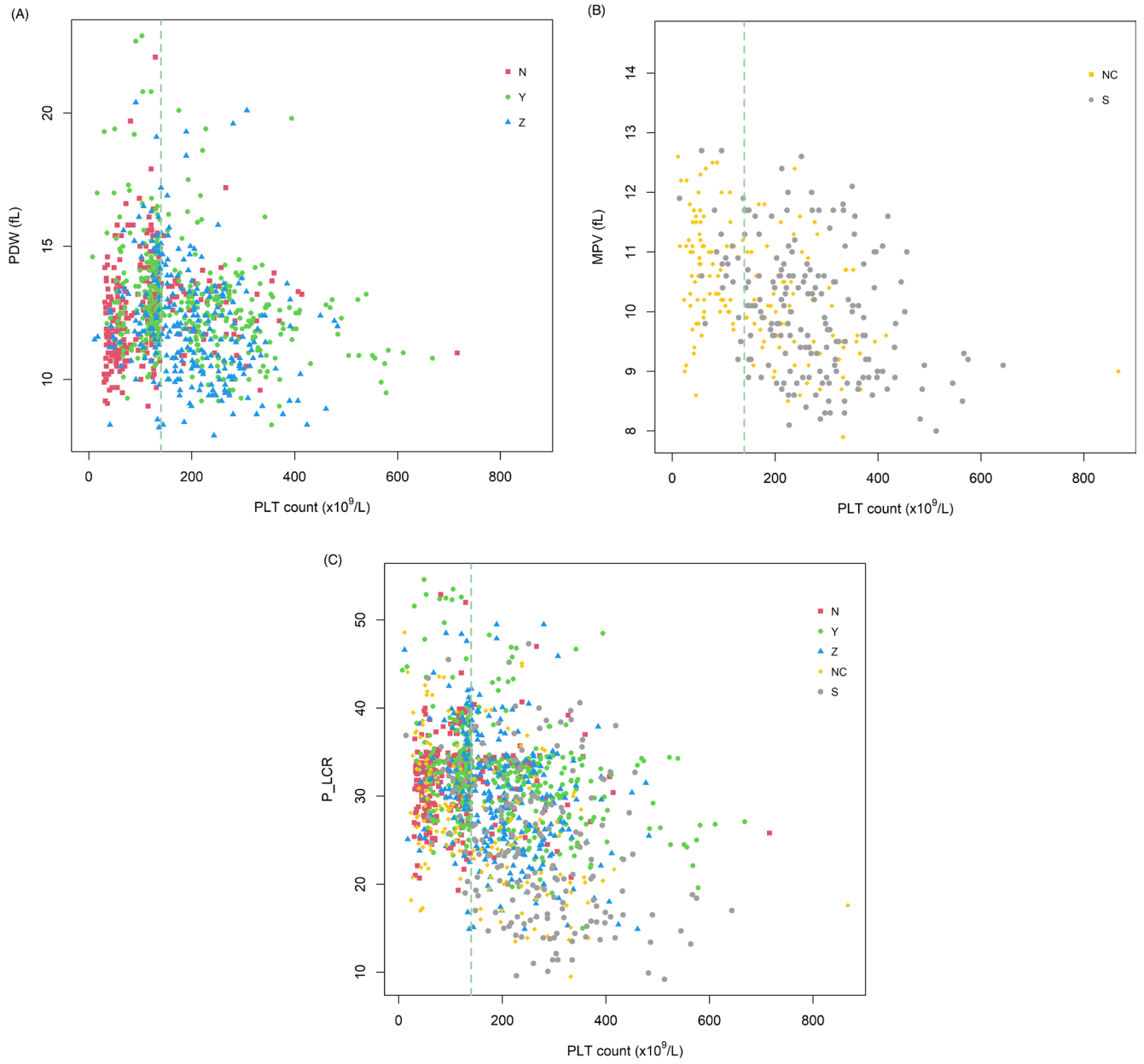

FIGURE 4 | Differences in platelet (PLT) count and indices among five groups. (A) PLT count and platelet distribution width (PDW) between groups N and Y and group Z: PDW of group Z was significantly lower than that of group N or Y. (B) PLT count and mean platelet volume (MPV) between group NC and group S: MPV of group S was significantly lower than that of group NC. (C) PLT count and platelet large cell ratio (P_LCR) among groups NC, N, Y, Z, and S: P_LCR of group Z or S was significantly lower than that of group N/Y or NC, respectively. *Dashed line meaning PLT count of 140×10^9/L shows that specimens with more than 140×10^9/L were also flagged (dots of group N, Y, and Z located on the right side of the line).

"PLT_clump?" However, most of small to medium-sized hospital laboratories utilize only a simple hematology analyzer without sufficient number of laboratory personnel to review blood smears microscopically. Besides, there is no experienced hematologist in most of small Korean hospitals who can manage and control the process. Therefore, there is a need to make an appropriate protocol to detect platelet clumping accurately. In addition, there has been no consensus about how to define a specimen with PC. Until recently several studies about PC have used arbitrary definitions. One researcher defined it as PA larger than a white blood cell. However, some researchers did not specify the definition employed in their studies [11–14]. The only definition suggested by an expert group is not based on an experiment [1]. Hence, we tried to establish a definition. Interestingly, PC encountered in clinical laboratories usually consisted of a small number of platelets. The number of clumps in a single microscopic field was small. Group A accounted for most of the blood smears containing PC.

What was more important was that the initial platelet count reported by the XN-9000 and the final count were significantly different for every group. Moreover, even for group A, the mean difference was 82.36×10^9/L, which could make a clinically meaningful difference in platelet count. A change from

thrombocytopenia to a normal count or from a normal count to thrombocytosis might remove or identify the need for bone marrow study and treatment.

Therefore, we should define PC by the presence of at least three rather than five attached platelets suggested by GFHC and prepare each clinical laboratory's own guideline fit for its role in a community. A reference laboratory should have a stricter protocol for detecting PC to prevent excessive examination or treatment for a patient. For instance, a 49-year old man without medical history was referred to our hospital because of an unexplained thromobocytopenia. His platelet count had been fluctuating for several years, usually between 100×10^9/L and 150×10^9/L in a local laboratory. In our laboratory, his blood smear revealed some small-sized PAs and his optical platelet count was about 250×10^9/L. It was understandable that the local laboratory did not perform microscopic review for a patient with mild thrombocytopenia or dismissed indistinct PCs. However, if our laboratory had been not fully aware of the importance of small PCs, his clinician might have decided to perform a bone marrow examination considering his age and past history, which would cause unacceptable harm to the patient.

4.2 | Part 2: Performance of PC Detection of Sysmex XN Series

Modern hematology laboratories can handle an enormous number of specimens thanks to an automated hematology analyzer. However, at the same time, not every specimen is reviewed by a human in modern laboratories. Regarding pseudothrombocytopenia, a specimen is only rechecked when its platelet count is lower than the laboratory's own criteria or it is flagged as "PLT_clump?" The problem is that each manufacturer of automated hematology analyzer uses a different individual algorithm for flagging which is not open to users. In addition, insufficient efforts have been made to comprehend the flagging algorithm for platelets probably because the reference range is very broad to help to obscure errors in measuring and mild to moderate thrombocytopenia does not usually require an immediate management. Therefore, we tried to evaluate the capacity of Sysmex XN-9000 for detecting PC.

The prevalence of PC was considerably high (43.4%, Figure 2) since we included all samples with PC even though they did not show thrombocytopenia. We collected specimens in a tertiary hospital where most of in and out-patients had underlying diseases. Above all, we used a very sensitive criterion for the presence of PC. The sensitivity (2.18%) of a "PLT_clump?" flag reported by the Sysmex XN-9000 using the impedance method was lower than a previous study (20% using Noklus criteria and 26% using GFHC criteria) [7]. As that study showed that the sensitivity of the flag was lower with the more sensitive definition for PC, we assumed that because we used even more sensitive definition the sensitivity we estimated was lower [7]. The specificity (98.3%) of the flag was similar with the previous study (96% using Noklus criteria and 94% using GFHC criteria) [7].

There was room for improvement in the sensitivity, specificity, PPV (41.5%), and NPV (56.5%) of a "PLT_clump?" flag reported by the Sysmex XN-9000. According to Sysmex, PC flag was tagged when particles are present in the specific area of the WNR scatter plot [15]. Furthermore, a previous study has asserted that only a specimen with <140000 platelets per microliter is flagged as "PLT_clump?" [6]. When samples with varying degrees of artificially induced PC and without PC were compared, MPV and P_LCR were higher in samples with severe PC than negative samples [6]. In our study, we analyzed the PLT count and indices of real-world samples and confirmed that samples with more than 140×10^9/L platelets per microliter were also flagged (Figure 3A,C). We could also identify group S to which samples not flagged despite they contained PC. This group had a characteristic of a high platelet count with low MPV and P_LCR that were even lower than those of group NC (Table 2, Figure 3B,C). Thus, we supposed that because the previous study used specimens with artificially induced PC, there were some traits different from the reality. For example, when PC was induced by adding ADP in a laboratory, PCs might be formed uniformly in an entire tube, which meant that samples with more than 140×10^9/L platelets only had paired platelets and small clumps, assuming that researchers had used specimens with an average platelet count. Moreover, we revealed that among specimens with PC flag, PDW and P_LCR of specimens containing microclots (group Z) were significantly lower than groups N and Y (Table 2, Figure 3A,C).

Considering that group S was very small and of little clinical significance, the high proportion of group Z (presence of microscopic fibrin clot: 27.5%) was supposed to contribute considerably to lowering the PPV, and it is a big waste of time and energy processing a sample of group Z which eventually needs to be resampled until it is microscopically reviewed. Thus we propose to introduce a new index using PDW and P_LCR to discriminate a specimen with microcolots from groups N and Y at an earlier stage by further studies. If group Z is filtered out by the XN-9000 more perfectly, its PPV and NPV would increase to 56.6% and 56.8%, respectively. To differentiate groups N from Y, PCT might be helpful. Regarding thrombocytopenic samples with PCs without a platelet-related flag, which constituted a minor portion of group S {mean (SD) PLT count: $265.77\ (112.05)\times10^9$/L}, more attention from clinicians would be sufficient for detection. Clinicians should be aware of PTCP and limits of the flagging system and order a peripheral blood smear test in case of a spurious thrombocytopenia without any related symptoms or history. In summary, though additional studies are needed to determine the cutoff value for each index, we suggest a new workflow for dealing a PC flagged specimen in a clinical laboratory using the XN series as follows. First of all, an education for clinicians about the possibility of PTCP and for all blood-collecting hospital personnel about the importance of sample mixing for the prevention of fibrin clot formation is essential. Second, when a specimen is flagged as PC, one can request re-sampling without microscopic review based on the value of PDW and P_LCR (for instance, 12.1 and 30; needed to be verified by further studies). Third, when one review microscopically the rest of flagged specimen, specimen with the value of PCT of more than 0.16 (needed to be verified by further studies) is required to be more rigorously reviewed to find the presence of PC of any size.

This study has some limitations. First, we did not consider the effect of various size of PAs and the relationship between size and the number of PAs. Second, we did not suggest any specific

number as a new index for screening of group Z. Finally, Fonio method rather than the counting with a fluorescence flow cytometer, a reference method for the enumeration of platelets [16, 17], was used to determine the final platelet count.

In conclusion, because even a small PA could have a clinical significance, a sensitive detection system should be implanted in every clinical laboratory. First of all, we suggest a definition of the significant PC as a clump of more than three platelets. Second, a new threshold value for platelet-related indices needs to be developed to improve the efficiency of the current platelet clumping detection system of automated hematology analyzer. For example, further studies for determining the cut-off value of PDW and P_LCR (Table 2) for distinguishing the specimens with fibrin clots from the specimens with platelet clumps without reviewing microscopically would be useful.

Author Contributions

Suhyeon Woo: performed research, analyzed and interpreted data, performed statistical analysis, and wrote the manuscript. **Bohyun Kim:** designed research, analyzed and interpreted data. **Nam Hun Heo:** analyzed and interpreted data. **Min-sun Kim** and **Young Ahn Yoon:** performed research. **Young-Jin Choi:** analyzed and interpreted data.

Acknowledgements

This work was supported by Soonchunhyang University Research Fund.

Ethics Statement

This study was approved by the institutional review board of Soonchunhyang University Cheonan Hospital with waiving informed consent (2022–04-013).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

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

References

1. V. Baccini, G. Franck, J. Hugues, et al., "Platelet Counting: Ugly Traps and Good Advice. Proposals From the French-Speaking Cellular Hematology Group (GFHC)," *Journal of Clinical Medicine* 9 (2020): 808.

2. B. Lardinois, J. Favresse, B. Chatelain, G. Lippi, and F. Mullier, "Pseudothrombocytopenia-a Review on Causes, Occurrence and Clinical Implications," *Journal of Clinical Medicine* 10 (2021): 594.

3. M. E. Mylonakis, L. Leontides, R. Farmaki, P. Kostoulas, A. F. Koutinas, and M. Christopher, "Effect of Anticoagulant and Storage Conditions on Platelet Size and Clumping in Healthy Dogs," *Journal of Veterinary Diagnostic Investigation* 20 (2008): 774–779.

4. B. Nicola, "EDTA-Dependent Pseudothrombocytopenia: A Clinical and Epidemiological Study of 112 Cases, With 10-Year Follow-Up," *American Journal of Hematology* 50 (1995): 103–109.

5. W. H. Choe, Y. U. Cho, J. D. Chae, and S. H. Kim, "Pseudothrombocytopenia or Platelet Clumping as a Possible Cause of Low Platelet Count in Patients With Viral Infection: A Case Series From Single Institution Focusing on Hepatitis a Virus Infection," *International Journal of Laboratory Hematology* 35 (2013): 70–76.

6. P. Xu, K. Fang, X. Chen, et al., "The Flagging Features of the Sysmex Xn-10 Analyser for Detecting Platelet Clumps and the Impacts of Platelet Clumps on Complete Blood Count Parameters," *Clinical Chemistry and Laboratory Medicine* 60 (2022): 748–755.

7. H. E. Lunde, A. N. Hjelmtvedt, and E. K. Amundsen, "The Diagnostic Accuracy of Sysmex XN for Identification of Pseudothrombocytopenia Using Various Thresholds for Definition of Platelet Aggregation," *International Journal of Laboratory Hematology* 44, no. 5 (2022): 854–860.

8. C. Tantanate, "Vortex Mixing to Alleviate Pseudothrombocytopenia in a Blood Specimen With Platelet Satellitism and Platelet Clumps," *Clinical Chemistry and Laboratory Medicine* 59, no. 5 (2021): e189–e191.

9. L. G. Gene, A. Amy, and C. Conrad, "Using a Vortex to Disaggregate Platelet Clumps," *Laboratoriums Medizin* 28, no. 10 (1997): 665–667.

10. S. Mannuß, "Influence of Different Methods and Anticoagulants on Platelet Parameter Measurement," *Journal of Laboratory Medicine* 44, no. 5 (2020): 255–272.

11. P. C. Bartels, M. Schoorl, and A. J. Lombarts, "Screening for Edta-Dependent Deviations in Platelet Counts and Abnormalities in Platelet Distribution Histograms in Pseudothrombocytopenia," *Scandinavian Journal of Clinical and Laboratory* 57 (1997): 629–636.

12. G. L. Gulati, A. Asselta, and C. Chen, "Using a Vortex to Disaggregate Platelet Clumps," *Laboratory Medicine* 1997, no. 28 (1997): 665–667.

13. B. J. Oh, D. Cho, S. J. Kee, et al., "Correction Algorithm of Pseudothrombocytopenia Due to Platelet Clumping," *Annals of Laboratory Medicine* 25 (2005): 373–378.

14. D. Cho, B. J. Oh, I. H. Kim, C. H. Jeong, and D. W. Ryang, "Correction of Platelet Count Using a Vortex in Pseudothrombocytopenia," *Annals of Laboratory Medicine* 23 (2003): 151–156.

15. Sysmex Corporation, *Xn Series (Xn-9000) Instructions for Use* (Kobe/Japan: Sysmex Corporation, 2015).

16. C. Briggs, P. Harrison, and S. J. Machin, "Continuing Developments With the Automated Platelet Count," *International Journal of Laboratory Hematology* 29 (2007): 77–91.

17. International Council for Standardization in Haematology and International Society for Laboratory Hematology, "Platelet Counting by the Rbc/Platelet Ratio Method: A Reference Method," *American Journal of Clinical Pathology* 115 (2015): 460–464.