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ORIGINAL ARTICLE

Evaluation of Platelet Function Detection Using Sequential Platelet Counting Method via Fluorescent Platelet Channel

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SUMMARY

Background: **The sequential platelet counting method (SPCM) for platelet function detection relies on impedance-based platelet counting, which may be affected by factors such as microcytes and red blood cell fragments. The Sysmex XN series automated hematology analyzer incorporates a fluorescent platelet channel (PLT-F) based on flow cytometry, offering enhanced specificity compared to impedance methods.**
Methods: **This study compared platelet aggregation function between light transmission aggregometry (LTA) and SPCM-PLTF in healthy individuals and clopidogrel-treated patients. The effects of platelet-poor plasma (PPP) and normal saline (NS) dilution on LTA results were also analyzed.**
Results: **SPCM-PLTF revealed significant differences in platelet aggregation between healthy and patient groups ($p < 0.0001$). Although LTA and SPCM-PLTF results differed statistically ($p < 0.0001$), they exhibited strong correlation ($r = 0.849$, $p < 0.0001$). PPP dilution progressively reduced platelet aggregation ($p < 0.05$), while NS dilution showed no significant effect.**
Conclusions: **SPCM-PLTF effectively monitors platelet function and correlates well with LTA, offering a viable alternative for clinical use.**
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KEYWORDS

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INTRODUCTION

Platelet function testing is essential for evaluating thrombotic or hemorrhagic risks and optimizing antiplatelet therapy [1]. Since its inception in the 1960s [2], light transmission aggregometry (LTA) has been widely regarded as the "gold standard" [3]. However, LTA is constrained by interference from hemoglobin, triglycerides, and manual processing steps that may activate platelets [4]. The PL-11 platelet analyzer (Innovo Medical) employs the sequential platelet counting method (SPCM), which utilizes electrical impedance to measure platelet counts in whole blood samples before and after the addition of aggregation agonists. By assessing the change in platelet quantity, this method serves as an in-

dicator of platelet aggregation function [5]. SPCM-DC (defined herein as impedance-based SPCM) mitigates certain limitations but remains susceptible to inaccuracies from cellular debris interference in impedance-based counting.

The Sysmex XN-series hematology analyzers utilize a proprietary fluorescent platelet channel (PLT-F) for platelet enumeration. This technology operates on the principle that nucleic acid-rich organelles within platelets specifically bind to oxazine fluorescent dyes, followed by multidimensional analysis of stained platelets through flow cytometry and laser scatter techniques, enabling accurate platelet identification and counting [6-8]. This study developed a manual protocol simulating the PL-11 analyzer's operational workflow, utilizing the PLT-F channel instead of impedance-based counting to quantify platelet changes for functional assessment (herein defined as SPCM-PLTF), with comparative evaluation against light transmission aggregometry (LTA) performed on the Telicongxin AG800 system, aiming to assess the feasibility and advantages of the SPCM-PLTF method.

MATERIALS AND METHODS

Clinical data

Thirty patients receiving clopidogrel (17 males, 13 females; excluded: aspirin or NSAID users) and 30 healthy controls (14 males, 16 females; normal liver/kidney function, blood routine, and coagulation profiles) were enrolled from January 2024 through March 2025. Three cubital venous blood samples were drawn from each participant into 3.2% sodium citrate anticoagulant tubes (9:1 blood-to-anticoagulant ratio). All samples were maintained at room temperature (20 - 25°C) and processed within 2 hours. Blood collection tubes were provided by Greena Biotechnology Co., Ltd.

Instruments and reagents

The following instruments and reagents were used:

AG800 Optical Aggregometer (Telicon, China).

Sysmex XN2000 Hematology Analyzer (Sysmex, Japan).

BY160C Centrifuge (Beijing Baiyang Medical Instruments).

SCI-M 96-Well Vortex Mixer (SCILOGEX, USA).

ADP: 150 μmol/L (LTA; Telicon) and 50 μmol/L (SPCM-PLTF; Nanjing Shenzhou Innova Medical).

Reagents: Flourocell PLT, CELLPACK DFL, and CELLPACK DCL (Sysmex).

Reaction tubes: Wuxi Medical Equipment Co., China.

Experimental procedures

LTA protocol

Platelet-rich plasma (PRP) was prepared by centrifuging blood at 160 × g (981 rpm) for 10 minutes, while platelet-poor plasma (PPP) was obtained at 2,000 × g (3,711 rpm) for 10 minutes (platelet count < 10 × 10^9/L). For LTA, 300 μL PRP or diluted PRP was added to a cuvette. ADP (final concentration: 5 μmol/L) was added, and maximum aggregation ratio (MAR, %) was measured using PPP as a blank.

SPCM-PLTF protocol

Baseline platelet counts were measured twice using the PLT-F channel of the Sysmex XN2000 hematology analyzer, and the mean value was recorded as the initial count. Subsequently, 25 μL of ADP (50 μmol/L) was added to 250 μL whole blood to achieve a final ADP concentration of 5 μmol/L. The mixture was gently aspirated and dispensed five times using a pipette tip positioned at the bottom of the tube, ensuring complete homogenization within 30 seconds. Following activation, platelet counts were dynamically performed every 80 seconds for three consecutive cycles under continuous vortex mixing (30 rpm) (Figure 1). The lowest platelet count observed post-ADP addition was used to calculate the maximum aggregation ratio (MAR) as follows:

$$\text{MAR}\ (\%) = \left(\frac{\text{Initial count} - \text{Lowest post-ADP count}}{\text{Initial count}}\right) \times 100$$

Dilution effects on LTA

PRP samples from healthy volunteers with platelet counts > 600 × 10^9/L were diluted with PPP or normal saline (NS) at ratios of 1 ×, 2 ×, and 3 ×, ensuring that the post-dilution platelet count was not lower than 200 × 10^9/L. Subsequently, 300 μL of the adjusted PRP was analyzed via light transmission aggregometry (LTA) as described in the methodology above.

Statistical analysis

Data were analyzed using GraphPad Prism 10. Continuous variables were expressed as mean ± SD. Normality was assessed via Kolmogorov-Smirnov test. Group comparisons used independent *t*-tests; correlations were evaluated via Pearson analysis ($p < 0.05$ significant).

RESULTS

Comparison between LTA and SPCM-PLTF

The healthy group (mean age: 39.5 ± 11.1 years) and patient group (mean age: 43.5 ± 5.8 years) showed no significant differences in age or gender ($p > 0.05$). Light transmission aggregometry (LTA) revealed significantly higher platelet aggregation rates (MAR) in healthy individuals (62.93 ± 8.42%) compared to clopidogrel-treated patients (44.82 ± 7.72%; $p < 0.0001$; Figure 2A). Similarly, SPCM-PLTF demonstrated a marked difference between groups (healthy: 55.56 ± 8.22% vs. patients: 38.30 ± 7.88%; $p < 0.0001$; Figure 2A).

Methodologically, LTA yielded higher overall MAR values (53.87 ± 12.15%) than SPCM-PLTF (46.93 ± 11.81%; $p < 0.01$, Cohen's $d = 0.59$) (Figure 2A). A correlation was observed between the two methods ($r =$

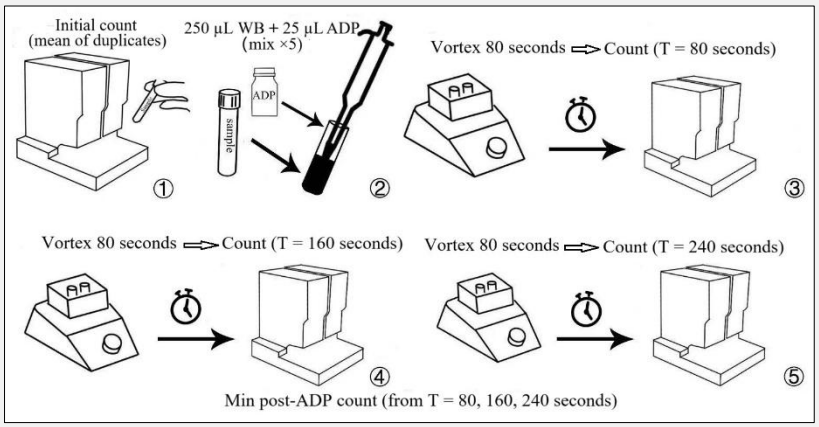

Figure 1. Schematic of the experimental operation.

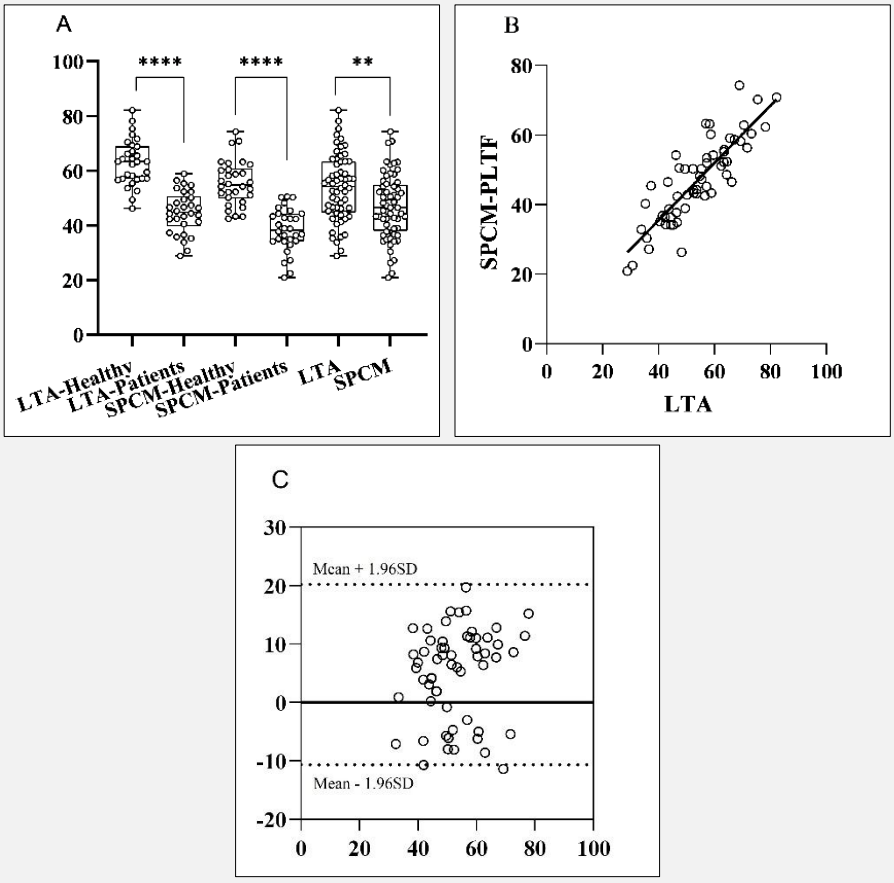

Figure 2. Comparison between LTA and SPCM-PLTF.

A **** $p < 0.0001$ for intergroup comparisons, ** $p < 0.01$ for intermethod comparisons (Cohen's $d = 0.59$). B Correlation analysis between LTA and SPCM-PLTF. A strong positive correlation was observed ($r = 0.849$, $p < 0.0001$). C Bland-Altman analysis showing the agreement between LTA and SPCM-PLTF. The dashed lines represent the 95% limits of agreement.

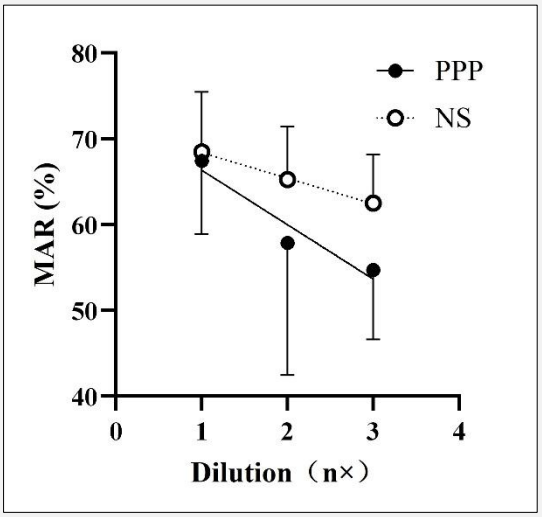

Figure 3. Effect of platelet count adjustment on platelet aggregation function.

Platelet aggregation rates (MAR) measured by LTA after diluting PRP with PPP or NS at ratios of 1 ×, 2 ×, and 3 ×. Data are presented as mean ± SD. $p < 0.05$ for PPP dilution, no significant differences were observed for NS dilution ($p > 0.05$).

0.849, $p < 0.0001$; Figure 2B), supported by Bland-Altman analysis (Figure 2C).

Impact of platelet count adjustment on aggregation function

In 18 healthy volunteer samples analyzed by LTA, platelet count adjustment using PPP resulted in progressively reduced MAR values with increasing dilution ratios: 67.41 ± 8.50% (1 ×), 57.41 ± 15.41% (2 ×), and 54.72 ± 8.09% (3 ×) ($p < 0.05$). Conversely, NS dilution maintained stable MAR across ratios (1 ×: 68.46 ± 7.00%; 2 ×: 65.26 ± 6.17%; 3 ×: 62.49 ± 5.65%; $p > 0.05$) (Figure 3).

DISCUSSION

Platelets, derived from megakaryocyte cytoplasmic fragments, exhibit unique morphological and biochemical characteristics critical for hemostasis and thrombosis. Antiplatelet drugs such as aspirin and clopidogrel inhibit platelet activation to manage thrombotic diseases. Accurate laboratory monitoring of antiplatelet therapy is essential to address interindividual variability in drug response and mitigate complications like bleeding. However, current platelet function assays lack a consensus "gold standard" due to methodological heterogeneity [9,10].

This study demonstrates that SPCM-PLTF effectively differentiates platelet aggregation between healthy individuals and patients, with significant intergroup differences in MAR. Furthermore, although larger cohorts of both healthy controls and patients are needed to establish stronger statistical correlations, SPCM-PLTF still shows consistency with LTA ($r = 0.849$). These findings support its validity for monitoring platelet function and therapeutic responses. LTA requires standardization of PRP platelet counts to 200 - 250 × 10^9/L using PPP [11], as platelet concentration directly influences sample turbidity. Comparative analysis revealed that PPP dilution progressively reduced MAR with increasing ratios (1 ×: 67.41% → 3 ×: 54.72%; $p < 0.05$), whereas NS dilution showed no significant effect (1 ×: 68.46% → 3 ×: 62.49%; $p > 0.05$). This suggests that platelet-activating substances are released from PPP during the centrifugation process. Dilution with PPP then introduces these activating substances into PRP [12,13], whereas SPCM circumvents this interference by eliminating manual count normalization. The LTA standardization threshold (200 - 250 × 10^9/L) may not be universally applicable. For instance, the normal platelet count in populations such as Sichuan, China, is approximately 150 × 10^9/L [14], and many laboratories consider PRP with counts > 100 × 10^9/L adequate for testing [15].

LTA standardization is further complicated by variables such as sample volume, plasma preparation, and detection timing. Moreover, pre-analytical count normalization may not fully replicate *in vivo* platelet behavior, underscoring the need for improved methodologies [16]. In contrast, SPCM-DC (impedance-based dynamic

counting method) minimizes manual steps and sample requirements while analyzing whole blood, which better mimics physiological conditions. SPCM also provides hematological parameters (RBC, PLT, MCV, MPV, e.g.) to identify confounders such as microcytes or platelet size abnormalities [5]. SPCM calculates MAR based on dynamic platelet count changes, necessitating high counting accuracy. Impedance-based methods (SPCM-DC) are prone to interference from cell fragments or microcytes, leading to overestimated counts, while microplatelets or platelet clumps may cause underestimation [7].

Beyond LTA and SPCM, clinically adopted devices for antiplatelet therapy monitoring include VerifyNow® and PFA-100®/200® systems. VerifyNow® utilizes turbidimetric analysis of whole blood with activator-coated microbeads, offering rapid point-of-care testing (3 - 5 minutes), standardized operation, and pathway-specific reporting (e.g. P2Y12 reaction units [PRU]). Limitations include restricted activator panels (ADP/AA/TRAP), absence of high-shear simulation, and elevated operational costs [17,18]. In contrast, PFA-100®/200® systems evaluate primary hemostasis via closure time (CT) measurement of capillary occlusion by platelet plugs under high-shear stress. While effective for von Willebrand disease (vWD) screening and detection of platelet disorders, CT results demonstrate hematocrit/platelet count dependency, low P2Y12 inhibitor sensitivity, and inability to differentiate defect mechanisms [19].The SPCM- PLTF method proposed in this study combines some of the advantages of these two types of instruments: similar to VerifyNow, it uses whole-blood testing to avoid the interference of centrifugation and simplifies the operation process. It is worth noting that methods such as LTA and SPCM directly quantify the dynamic platelet aggregation rate, and the dimension of their detection is essentially different from the closure-time assessment of PFA-100.

Future SPCM-PLTF automation could leverage its high platelet counting accuracy [6,20] to provide more reliable MAR measurements. This offers a promising monitoring solution, combining PLT-F accuracy, clinical practicality (no standardization needed), and efficiency-especially for samples with interferences like microcytosis. However, it requires fresh whole blood for accurate counts and currently has longer processing times and higher reagent costs than impedance methods.

In this study, LTA consistently yielded higher MAR values than SPCM-PLTF, likely due to methodological differences (plasma vs. whole blood) and manual operational variability.

CONCLUSION

SPCM-PLTF effectively reflects platelet function and monitors post-treatment changes. It shows a correlation with LTA and provides a standardized approach to assess platelet function. Automation and integration with impedance-based counting could further enhance its clinical utility.

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Ethical Approval Statement:
This study was approved by the Ethics Committee of the First Affiliated Hospital of Hebei North University in January 2024 (approval number: K2024080). Informed consent was obtained from all participants in writing prior to sample collection.

Data Availability Statement:
The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Declaration of Interest:
The authors declare no potential conflicts of interest.

References:

1. Bolliger D, Lancé MD, Siegemund M. Point-of-Care Platelet Function Monitoring: Implications for Patients With Platelet Inhibitors in Cardiac Surgery. J Cardiothorac Vasc Anesth 2021; 35(4):1049-59. (PMID: 32807601)

2. Salzman EW, Chambers DA, Neri LL. Possible mechanism of aggregation of blood platelets by adenosine diphosphate. Nature 1966;210(5032):167-9. (PMID: 5962074)

3. Le Blanc J, Mullier F, Vayne C, Lordkipanidzé M. Advances in Platelet Function Testing-Light Transmission Aggregometry and Beyond. J Clin Med 2020;9(8):2636. (PMID: 32823782)

4. Mylotte D, Foley D, Kenny D. Platelet function testing: methods of assessment and clinical utility. Cardiovasc Hematol Agents Med Chem 2011;9(1):14-24. (PMID: 21121898)

5. Guan J, Cong Y, Ren J, et al. Comparison between a new platelet count drop method PL-11, light transmission aggregometry, VerifyNow aspirin system and thromboelastography for monitoring short-term aspirin effects in healthy individuals. Platelets 2015; 26(1):25-30. (PMID: 24433273)

6. Seo JY, Lee ST, Kim SH. Performance evaluation of the new hematology analyzer Sysmex XN-series. Int J Lab Hematol 2015; 37(2):155-64. (PMID: 24815300)

7. Baccini V, Geneviève F, Jacqmin H, et al. Platelet Counting: Ugly Traps and Good Advice. Proposals from the French-Speaking Cellular Hematology Group (GFHC). J Clin Med 2020;9(3): 808. (PMID: 32188124)

8. Tanaka Y, Tanaka Y, Gondo K, et al. Performance evaluation of platelet counting by novel fluorescent dye staining in the XN-series automated hematology analyzers. J Clin Lab Anal 2014; 28(5):341-8. (PMID: 24648166)

9. Gurbel PA, Becker RC, Mann KG, Steinhubl SR, Michelson AD. Platelet function monitoring in patients with coronary artery disease. J Am Coll Cardiol 2007;50(19):1822-34. (PMID: 17980247)

10. Bonello L, Camoin-Jau L, Arques S, et al. Adjusted clopidogrel loading doses according to vasodilator-stimulated phosphoprotein phosphorylation index decrease rate of major adverse cardiovascular events in patients with clopidogrel resistance: a multicenter randomized prospective study. J Am Coll Cardiol 2008;51(14): 1404-11. (PMID: 18387444)

11. Christie DJ, Avari T, Carrington LR, et al. Platelet function testing by aggregometry; approved guideline. In, Vol. 2024, 2011. https://fritsmafactor.com/content/user_files/sites/3/2011/04/h58-a.pdf

12. Harrison P, Segal H, Blasbery K, Furtado C, Silver L, Rothwell PM. Screening for aspirin responsiveness after transient ischemic attack and stroke: comparison of 2 point-of-care platelet function tests with optical aggregometry. Stroke 2005;36(5):1001-5. (PMID: 15817896)

13. Cattaneo M, Lecchi A, Zighetti ML, Lussana F. Platelet aggregation studies: autologous platelet-poor plasma inhibits platelet aggregation when added to platelet-rich plasma to normalize platelet count. Haematologica 2007;92(5):694-7. (PMID: 17488697)

14. Wu X, Zhao M, Pan B, et al. Complete blood count reference intervals for healthy Han Chinese adults. PLoS One 2015;10(3): e0119669. (PMID: 25769040)

15. Cattaneo M, Hayward CP, Moffat KA, Pugliano MT, Liu Y, Michelson, AD. Results of a worldwide survey on the assessment of platelet function by light transmission aggregometry: a report from the platelet physiology subcommittee of the SSC of the ISTH. J Thromb Haemost 2009;7(6):1029. (PMID: 19422455)

16. Tantry US, Gurbel PA. Assessment of oral antithrombotic therapy by platelet function testing. Nat Rev Cardiol 2011;8(10):572-9. (PMID: 21769112)

17. Michelson AD, Frelinger AL 3rd, Furman MI. Current options in platelet function testing. Am J Cardiol 2006;98(10A):4N-10N. (PMID: 17097417)

18. van Werkum JW, Harmsze AM, Elsenberg EH, Bouman HJ, ten Berg JM, Hackeng CM. The use of the VerifyNow system to monitor antiplatelet therapy: a review of the current evidence. Platelets 2008;19(7):479-88. (PMID: 18979359)

19. Harrison P, Robinson M, Liesner R, et al. The PFA-100: a potential rapid screening tool for the assessment of platelet dysfunction. Clin Lab Haematol 2002;24(4):225-32. (PMID: 12181026)

20. Hummel K, Sachse M, Hoffmann JJML, van Dun LPJM. Comparative evaluation of platelet counts in two hematology analyzers and potential effects on prophylactic platelet transfusion decisions. Transfusion 2018;58(10):2301-8 (PMID: 30222860)