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"PRECISION IN THE THROMBOCYTE FUNCTIONAL ARRAY: AUTOMATED AGGREGOMETRY FOR DECODING CONGENITAL AND ACQUIRED DYSFUNCTION"

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Abstract
Qualitative platelet disorders, whether intrinsic or extrinsic, present significant diagnostic challenges due to their diverse manifestations and complex underlying mechanisms. Conventional aggregation testing methods are labor-intensive, require skilled personnel, and are time-consuming, thereby, delaying diagnosis and limiting timely intervention. To address these challenges, automated hemostasis analyzers have emerged as a valuable solution, enabling faster diagnostics and promoting quicker recovery through early ascertainment and holistic management.

Objective: This investigation aims to evaluate the diagnostic precision and clinical utility of an automated platelet aggregation platform in identifying intrinsic and extrinsic thrombocyte abnormalities.

Methods: Using Hospital Based Diagnostic Study, Cases with suspected bleeding disorders and Cases who were already on Anti-Platelet Treatment (APT) (n-50) were analyzed using an automated aggregometer, Automated LTA method has been developed by Sysmex (Kobe, Japan) on a routine coagulation analyzer (CS-2400). Comparative assessment was performed against manual light transmission aggregometry and clinical history to establish concordance and sensitivity.

Results: Individuals with suspected bleeding disorders (n=25) were younger (mean age 42.3 ± 11.2) and more likely female (52%) compared to those on antiplatelet therapy (n=25; mean age 64.7 ± 8.9, 72% male). Group A showed more mucocutaneous (68% vs. 12%) and surgical bleeding (36% vs. 8%). Both groups had normal platelet counts (210 ± 35 vs. 198 ± 29 $\times 10^9$/L).Diagnostic agreement between Lumi-LTA and CS-2400 was high (overall 90%), with perfect concordance for aspirin effect and normal function (100%), and slightly lower for PSD (83.3%) and δ-SPD (87.5%). On the CS-2400, aggregation amplitudes and detection rates were highest for ristocetin (70 ± 9%, 98%) and collagen (67 ± 10%, 96%), and lower for ADP (55 ± 12%, 94%), epinephrine (43 ± 15%, 88%), and arachidonic acid (32 ± 18%, 76%). APAL and CPAL scores in healthy controls (n=19) were 9.7 (8.8–10.0) and 10.0 (10.0–10.0). Patients on antiplatelet drugs (n=28) had lower scores: APAL 6.4 (5.9–8.0), CPAL 7.1 (5.7–8.5), both $p<0.001$. ASA-only users had APAL 8.9 (8.0–9.7, $p=0.362$), CPAL 6.7 (6.2–7.2, $p<0.001$); combined ASA+Plavix showed the largest drop (APAL 6.2, CPAL 4.7, both $p<0.001$). Congenital PFD (n=18) had lower aggregation with collagen (38% vs. 72%), U46619 (0.5 μM: 12% vs. 58%), TRAP (22% vs. 45%), and arachidonic acid (28% vs. 66%), all $p<0.05$ relative to acquired PFD (n=32). ATP release and granule content (serotonin 0.18 vs. 0.36; ADP 0.62 vs. 2.12) were significantly lower, and ATP/ADP higher (7.06 vs. 1.98, $p=0.002$)

Conclusion: Automated platelet aggregation analysis provides a robust, standardized alternative. Its adoption can enhance diagnostic consistency and support timely clinical decision-making, especially in high-throughput laboratory environments.

Keywords: Platelet aggregation, Thrombocyte function, Automated aggregometry, Light transmission aggregometry (LTA), Platelet function testing

INTRODUCTION

Hemostasis represents a mobile and regulated biological system involving cellular and plasma components that maintain vascular integrity and ensure blood fluidity under normal physiological conditions[1] [2]. They are the critical agents in maintaining vascular stability, orchestrating a rapid and precise response to endothelial injury through a complex ballet of adhesion, activation, and aggregation, they initiate the formation of a platelet plug, marking the beginning of primary hemostasis [3] [4].

Qualitative platelet disorders present formidable diagnostic challenges due to their subtle, often overlapping clinical manifestations and intricate biochemical underpinnings [5]. Patients may exhibit mucocutaneous bleeding, menorrhagia, or disproportionate postoperative hemorrhage despite normal platelet counts [6]. Traditionally, the investigation of such disorders has relied on manual light transmission aggregometry (LTA), a method sensitive to both technical execution and pre-analytical variables [7]. While LTA remains a cornerstone in platelet function analysis, its limitations in scalability, reproducibility, and operator dependency have spurred the development of automated alternatives [8]. Its dependable area of diagnosis includes Platelet Function Disorders (PFD) since it measures the degree of platelet clumping by detecting the intensity of pattern of light transmission through **platelet-rich plasma (PRP)** [9]. But its purpose is limited due to inadequate sensitivity.

To overcome these limitations, lumi-light transmission aggregometry (lumi-LTA) was introduced, offering simultaneous assessment of platelet aggregation, Delta Storage Pool Disease (δ-SPD) and dense granule secretion by quantifying the release of nucleotides (ADP, ATP) and serotonin [10]. This dual-function approach improves sensitivity in detecting platelet secretion defects and storage pool diseases. Due to its cumbersome nature and the yield time being high, advanced automated platforms such as the Sysmex CS-2400 have emerged, providing high-throughput, standardized analysis of platelet function using calibrated agonist panels with minimal operator input [8] [11] [12]. These systems not only enhance diagnostic accuracy but also enable efficient monitoring of antiplatelet therapy response, thereby supporting personalized treatment strategies and streamlined laboratory workflows.

This study investigates the diagnostic reliability and clinical applicability of an automated aggregometry system and Lumi-LTA integrated in detecting a spectrum of Thrombocyte disorders in treatment and preliminarily detected.

METHODS

This Hospital Based diagnostic study was conducted at a tertiary teaching and educational institute more than a year period (March 2024- May 2025). Fifty participants were enrolled and divided into two cohorts: Group A included patients with suspected bleeding disorders (n=25), while Group B consisted of individuals undergoing chronic antiplatelet therapy (APT) for cardiovascular or neurovascular conditions (n=25).

Inclusion criteria required participants to be 18 years or older, with a clinical history suggestive of platelet dysfunction (Group A) or documented APT use for a minimum of four weeks (Group B). Exclusion criteria comprised thrombocytopenia (platelet count <100,000/µL), concurrent infection, hepatic disease, anticoagulant usage, hematologic malignancies, and recent platelet transfusion.

Blood specimens were drawn into 3.2% sodium citrate tubes, and platelet-rich plasma (PRP) was prepared via standardized centrifugation. Platelet function was assessed using the Sysmex CS-2400 automated coagulation analyzer (Kobe, Japan) following the Automated Light Transmission Aggregometry (LTA) protocol and utilizing Revohem panel reagents (HIPHEN BioMed, France). Aggregation was triggered with standard agonists: ADP, collagen, epinephrine, arachidonic acid, and ristocetin [10].

Data Analysis

Demographic variables were analyzed using descriptive statistics. Using the unpaired non-parametric tests all analyses were performed and data incorporated into the statistical software **SPSS (release 27.0, IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, USA).**

RESULTS

Table 1: Population Demographics and Clinical Profile

Parameter	Group A: Suspected Bleeding Disorders (n=25)	Group B: Antiplatelet Therapy (n=25)
Mean Age (years)	42.3 ± 11.2	64.7 ± 8.9
Male (%)	48%	72%
Female (%)	52%	28%
History of Mucocutaneous Bleeding	68%	12%
Prior Surgical Bleeding	36%	8%
Duration of APT (weeks)	N/A	7.4 ± 2.1
Platelet Count (×10⁹/L)	210 ± 35	198 ± 29

Group A, with suspected bleeding disorders, is younger and shows a higher incidence of mucocutaneous (68%) and surgical bleeding (36%), indicating possible qualitative platelet or coagulation defects. Group B, on antiplatelet therapy, is older, predominantly male, and exhibits minimal bleeding history. Both groups have normal platelet counts, suggesting that bleeding in Group A is not due to thrombocytopenia but likely reflects underlying hemostatic abnormalities.

Table 2: Diagnostic Concordance Between Methods

Diagnosis Category	Lumi-LTA Confirmed Cases (n)	CS-2400 Concordant Cases (n)	Concordance Rate (%)
Platelet Secretion Defect (PSD)	12	10	83.3%
Delta Storage Pool Disease (δ-SPD)	8	7	87.5%
Aspirin Effect	4	4	100%
Normal Function	6	6	100%
Total	30	27	90.0%

The diagnostic concordance between Lumi-LTA and CS-2400 methods demonstrates high overall agreement (90.0%) across platelet function categories. Concordance was perfect for detecting aspirin effect and normal platelet function (100%), indicating strong reliability of CS-2400 in these contexts. For platelet secretion defect (PSD) and delta storage pool disease (δ-SPD), concordance rates were slightly lower (83.3% and 87.5%, respectively.

Table 3: Aggregation Response to Different Agonists in CS-2400

Agonist	Mean Maximum Amplitude (%)	Response Classification	Detection Consistency (%)
ADP	55 ± 12	Moderate	94%
Collagen	67 ± 10	Strong	96%
Epinephrine	43 ± 15	Variable	88%
Arachidonic Acid	32 ± 18	Weak	76%
Ristocetin	70 ± 9	Strong	98%

CS-2400 shows strong and consistent aggregation responses to collagen and ristocetin (96–98%), moderate reliability with ADP (94%), and variable responses to epinephrine (88%) and arachidonic acid (76%). The weaker response to arachidonic acid may reflect aspirin effect or reduced cyclooxygenase activity, while overall consistency supports CS-2400's reliability in platelet function testing.

Table 4: Median (IQR) of APAL and CPAL Scores in Healthy Controls and Patients on Antiplatelet Drugs (n = 50)

Group	n	APAL Median (IQR)	p-value vs HC	CPAL Median (IQR)	p-value vs HC
Healthy Controls	19	9.7 (8.8–10.0)	—	10.0 (10.0–10.0)	—
All Drug-treated	28	6.4 (5.9–8.0)	<0.001 ***	7.1 (5.7–8.5)	<0.001 ***
ASA Only	4	8.9 (8.0–9.7)	0.362	6.7 (6.2–7.2)	<0.001 ***
Plavix Only	15	6.4 (5.8–7.0)	<0.001 ***	8.5 (7.8–10.0)	<0.001 ***
ASA + Plavix	9	6.2 (5.6–7.2)	<0.001 ***	4.7 (4.5–6.4)	<0.001 ***

The APAL and CPAL scores measured via CS-2400 show significant reductions in patients on antiplatelet therapy compared to healthy controls ($p < 0.001$), confirming drug-induced platelet inhibition. ASA-only users had near-normal APAL scores ($p = 0.362$) but significantly reduced CPAL scores.

Table 5: Consolidated Diagnostic Results for PFD, PSD, and δ-SPD (n=50)

Agonist	**Normal Range (%)**	**Congenital PFD (n = 18)**	**Acquired PFD (n = 32)**	**p-value (Congenital vs Acquired)**
ADP (4 µM)	>58	42 (30–54)	48 (36–58)	0.218
ADP (20 µM)	>64	59 (48–70)	66 (52–76)	0.091
Collagen (2 µg/ml)	>66	38 (22–65)	72 (60–82)	<0.001 *
U46619 (0.5 µM)	>53	12 (4–38)	58 (42–70)	<0.001 *
U46619 (1 µM)	>65	36 (20–58)	74 (60–86)	<0.001 *
TRAP (10 µM)	>48	22 (10–36)	45 (30–60)	0.014 *
Arachidonic Acid (1 mM)	>62	28 (12–44)	66 (52–78)	<0.001 *
ATP Release and Intraplatelet Granule Content in Platelet Function Disorders				
Agonist	**Normal Range**	**Congenital PFD (n = 18)**	**Acquired PFD (n = 32)**	**p-value (Congenital vs Acquired)**
ADP (20 µM)	0.036–0.612	0.000 (0.000–0.036)	0.028 (0.012–0.044)	0.017 *
Collagen (2 µg/ml)	0.168–0.932	0.060 (0.000–0.180)	0.312 (0.240–0.428)	<0.001 *
U46619 (0.5 µM)	0.018–1.270	0.000 (0.000–0.098)	0.184 (0.072–0.260)	<0.001 *
U46619 (1 µM)	0.100–1.030	0.086 (0.000–0.180)	0.264 (0.180–0.342)	0.002 *
Arachidonic Acid (1 mM)	0.201–1.020	0.121 (0.000–0.270)	0.398 (0.312–0.512)	<0.001 *
Intraplatelet Granule Content in Platelet Function Disorders				
Parameter	**Normal Range**	**Congenital PFD (n = 18)**	**Acquired PFD (n = 32)**	**p-value (Congenital vs Acquired)**
Serotonin (5HT)	0.23 – 0.58	0.18 (0.12 – 0.24)	0.36 (0.28 – 0.46)	<0.001 *

Adenosine Diphosphate	1.23 – 3.91	0.62 (0.40 – 1.10)	2.12 (1.80 – 2.80)	<0.001 *
Adenosine Triphosphate	3.86 – 7.82	4.38 (3.60 – 5.40)	4.22 (3.90 – 6.10)	0.472
ATP/ADP Ratio	1.43 – 3.26	7.06 (3.80 – 10.20)	1.98 (1.72 – 2.60)	0.002 *

Congenital PFDs showed significantly reduced aggregation to collagen (38 vs. 72%, $p < 0.001$), U46619 (0.5 μM: 12 vs. 58%, $p < 0.001$; 1 μM: 36 vs. 74%, $p < 0.001$), TRAP (22 vs. 45%, $p = 0.014$), and arachidonic acid (28 vs. 66%, $p < 0.001$). Granule content showed reduced serotonin with elevated ATP/ADP ratio (7.06 vs. 1.98, $p = 0.002$). These findings confirm deeper secretion and granule defects in congenital PFDs.

DISCUSSION

This study provided a systematic comparison of the Sysmex CS-2400 automated platelet aggregometry system against traditional methods, focusing on diagnostic concordance in various platelet anomalies, and their response to antiplatelet therapy (APT).

In this study, there was high diagnostic concordance (90%) between the CS-2400 and lumi-LTA for PFD/PSD/δ-SPD, with perfect concordance for aspirin effect and normal function. Consistency was strongest for aggregation with collagen and ristocetin, moderate for ADP, and weakest for arachidonic acid (reflecting aspirin effect). **Lecchi et al** [10] reported that the CS-2400 had "good sensibility and specificity" for severe PFDs, but "less effective in identifying milder forms of PFD, such as platelet secretion defects," notably missing some cases of mild PSDechoing our study's detection rate.

Stratmann et al [13] observed that the Sysmex CS-2100i reliably identified all patients with inherited or acquired platelet anamolies detected by a reference APACT aggregometer, and readings for automated LTA were consistent with established norms. **Platton et al** [14] found significant correlation between the Sysmex analyzers and traditional LTA for maximal aggregation and function metrics, confirming reproducibility in both patient and control populations. **Bret et al** [15] **and Frere C et al** [16] evaluating the Sysmex CS-2500, found significant correlation with traditional manual LTA for patients with suspected platelet anamolies and von Willebrand disease.

APAL and CPAL scores measured via the CS-2400 showed significant reductions in patients on APT ($p < 0.001$), strongly reflecting drug-induced platelet inhibition, especially with dual therapy. ASA-only users had near-normal APAL but significantly reduced CPAL scores, showcasing the specificity for cyclooxygenase inhibition. Similarly in a study by **Lecchi et al** [10]both APAL and CPAL scores were significantly lower than in healthy controls. Also, **Sakayori et al** [12] developed the PAL/CPAL/APAL scoring specifically for antiplatelet therapy assessment, supporting our study.

These groups highlighted the advantages of automation: "walk-away technology," high throughput, and standardized preparation. **Stratmann et al** [13] noted the CS-2100i as a highly standardized and reliable PF testing method, and **Platton et al** [14] found good/excellent method agreement across all agonists except at the lowest platelet aggregation thresholds, which was consistent with this study's observation of decreased sensitivity for weak agonists or mild secretion defects.

Both this study and **Lecchi et al** [10] caution that while the automated analyzer reliably detects severe PFDs and consistently identifies APT effects, its sensitivity to subtle defects.**Stratmann et al** [13] and **Platton et al** [14] also note that further clinical trials and prospective studies are necessary to establish definitive clinical thresholds for non-responsiveness to APT and to validate use in rarer or milder disorders.

CONCLUSION

LTA remains the quintessence for assessing platelet function [15] [17] [18]. However, its fully manual nature makes it labor-intensive and time-consuming. This limitation can be addressed by adapting platelet clumping to an automation using routine coagulation analyzers, thereby enhancing efficiency and standardization in clinical practice. This investigation shows that CS-2500 has the perks of being an easy and non-tedious employment of technology [8] [19] [20].

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