Evaluation of an automated platelet aggregation method for detection of congenital or acquired platelet function defects

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Background - Light transmission aggregometry (LTA) is the most widely used laboratory method for an initial screening of patients with a suspected platelet function defect (PFD), and its use has also been proposed for assessing the efficacy of antiplatelet treatment (APT). An automated LTA method has been developed by Sysmex (Kobe, Japan) on a routine coagulation analyzer (CS-2400), together with a new research parameter called PAL (platelet aggregation level) to evaluate patients on APT.

Materials and methods - We evaluated the performance of CS-2400 compared to a stand-alone lumi-dual-aggregometer device in the diagnosis of PFD and in assessing the efficacy of APT. For these purposes, the study population was represented by a cohort of 23 patients with a previous diagnosis of PFD and a cohort of 28 patients on APT.

Results - Compared to healthy volunteers, patients with PFD showed a statistically significant reduction ($p<0.05$) in the maximal %light transmission, irrespective of the agonist used, both with the CS-2400 and the lumi-dual-aggregometer. As regards PFD patients, CS-2400 was effective in identifying the more severe defects, with a good sensibility and specificity, but less effective in identifying milder forms of PFD, such as platelet secretion defects. Patients on APT showed a statistically significant ($p=0.001$) reduced median %light transmission and PAL scores compared to healthy controls.

Discussion - Thanks to this LTA technology, CS-2400, a routine coagulation analyzer widely available in routine laboratories, could prove useful for initial assessment of patients with a suspected PFD. Moreover, the PAL scores were a fairly accurate reflection of the platelet response to APT.

Keywords: *primary hemostasis, platelet aggregation, light transmission aggregometry, platelet function defect, antiplatelet treatment.*

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INTRODUCTION

Hemostasis is a complex biological system involving finely balanced cellular and humoral mechanisms which in normal conditions, preserves blood fluidity and blood vessel integrity and provides prompt control of bleeding in case of vascular injury by clot formation. Primary hemostasis is the first of two steps that leads to the formation

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of a weak platelet plug and involves a close platelet-vessel interplay[1-3].

Inherited or acquired abnormalities of platelet function are associated with an increased risk of bleeding, proving that platelets play an important role in hemostasis. Typically, patients with platelet disorders have mucocutaneous bleedings of variable severity and disproportionate hemorrhage after surgery or trauma[4].

Light transmission aggregometry (LTA) is the most widely used method of studying platelet function during the initial screening of patients with a suspected platelet function disorder (PFD). LTA measures the transmission of light through a sample of platelet-rich plasma (PRP), which increases when platelets are induced to aggregate by different agonists. Due to its suboptimal sensitivity, a modification of traditional LTA has been developed, namely lumi-light transmission aggregometry (lumi-LTA), which measures platelet aggregation and secretion simultaneously[5]. After a diagnosis of PFD with lumi-LTA has been made, it is essential to measure the delta granule nucleotides (adenosine diphosphate [ADP], adenosine triphosphate [ATP]) and serotonin (5HT) content, so as to distinguish between a platelet secretion defect (PSD), characterized by a signaling pathway disorder leading to granule release defects, and a delta storage pool disease (δ-SPD), characterized by exhausted granule content, due to a deficiency in delta granule biogenesis[6].

The use of platelet function tests has also been advocated to evaluate residual platelet activity as a possible predictor of thrombotic recurrence in patients with previous arterial thrombotic events while on antiplatelet treatment (APT)[7].

LTA is a time-consuming and technically challenging method affected by many pre-analytical and analytical variables, which must be carefully controlled for by experts. Introducing automated devices could reduce the variability of the method. Recently, new automated coagulation analyzers, such as CS-2400 (Sysmex, Kobe, Japan) have made it possible to perform routine coagulation tests and platelet aggregation analysis on one single instrument[8,9].

To date, only a few studies have evaluated the performance of CS-2400 series automated analyzers in platelet aggregation. Two of those included patients with suspected bleeding disorders but in those two studies, only one[10] and three[11] patients, respectively, proved to actually have a PFD, while the other two studies considered patients with suspected platelet function defects that were not better specified[12,13]. A few other studies have been conducted only on healthy volunteers to identify the correct agonist concentrations[14] or to develop specific scores used for APT activity evaluation[15].

This study aims to compare the results obtained using an LTA method applied in an automated high-performance coagulation system, CS-2400, and in a stand-alone device lumi-dual-aggregometer (Chronolog, Mascia-Brunelli, Milano, Italy), in a cohort of patients previously diagnosed with a PFD (PSD or δ-SPD) at our tertiary referral center and in a cohort of patients on APT. The primary outcome of the study was the evaluation of the diagnostic capacity of CS-2400 in combination with Revohem aggregation panel reagents (HIPHEN BioMed S.A.S., Neuville-sur-Oise, France) in patients with a previous diagnosis of congenital or acquired PFD. The secondary outcome was the evaluation of the usefulness of CS-2400 in monitoring the effects of the drug regimen in a cohort of patients on chronic daily treatment with APT.

MATERIAL AND METHODS

Study design and population

This is a cross-sectional study performed on a historical cohort of patients followed at our center, either for a PFD or because they received treatment with antiplatelet drugs between January 1st 2020 and December 31st 2021.

The PFD had been diagnosed in patients with a history of bleeding when the platelet count was normal, together with the blood clotting screening (prothrombin time, activated partial thromboplastin time, fibrinogen) and the von Willebrand factor screening (von Willebrand factor antigen, ristocetin cofactor activity and factor VIII coagulant activity). To confirm the diagnosis of PFD, both aggregation and secretion were evaluated by lumi-LTA with a method based on the bioluminescent determination of ATP released from platelet δ-granules, as previously described[5]. To characterize the PFD as PSD or δ-SPD, the diagnostic process was then completed by measuring the platelet intragranular content of ATP, ADP, and serotonin as previously described[16,17].

The cohort on APT was represented by those patients receiving daily treatment with ASA, clopidogrel, or both.

A group of subjects with no personal or family history of

hemorrhage/thrombosis, with normal platelet aggregation and secretion, and not receiving APT or any drug known to interfere with platelet function during the previous 10 days, were used as healthy controls (HC) during the same study period.

The study was approved by the Ethic Committee of Milano Area 2 and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participating subjects.

Blood sampling

For platelet function studies, blood samples were collected from the antecubital vein using a 21-gauge butterfly needle and a tourniquet which was released soon after needle insertion. Thirty or 20 mL of whole blood (for our primary and secondary aim respectively) were collected in home-made tubes containing trisodium citrate 129 mmol/L (1/10 volumes).

To measure hematological parameters and immature platelets fraction (IPF%), whole blood samples were collected in K-EDTA tubes (Sarstedt, Verona, Italy) and analyzed in a Sysmex XN 1000 hematology analyzer (Sysmex) equipped with a fluorescence channel for platelets counting.

Platelet aggregation evaluation

In accordance with ISTH recommendations, PRP or PPP were obtained by centrifugation of citrated blood at room temperature at 200 × *g* for 10 min or 1,400 × *g* for 15 min, respectively[18]. The platelet counts in the PRP samples were not adjusted to a predefined value[19].

LTA testing was simultaneously conducted for both assays and completed within 4 hours after blood sampling.

In the Chronolog aggregometer, platelet aggregation is performed at 37°C and at 1,000 rpm. After 1 min of incubation at 37°C without stirring, the agonists are manually added to 450 µL of PRP. The agonists routinely used in our laboratory for diagnostic workup were used for platelet aggregation (ADP 2, 4 and 20 µM; arachidonic acid, AA 1 mM; epinephrine 5 µM [Sigma-Aldrich, St. Louis, MO, USA]; collagen 2 µg/mL [Horn Collagen, Mascia Brunelli]) and for the evaluation of APT efficacy (ADP 2, 4 and 20 µM; arachidonic acid, AA 1 mM [Sigma-Aldrich]).

In the CS-2400 analyser, platelet aggregation is carried out at 37°C and at 800 rpm, as recommended. A quantity of 140 µL of PRP is incubated at 37°C for 30 seconds and different agonists are automatically added to the sample. A Revohem aggregation panel reagents were used for platelet aggregation (ADP 2, 4 and 20 µM; arachidonic acid 1 mM; epinephrine 5 µM; collagen 2 µg/mL) and for the evaluation of APT efficacy (ADP 4 and 20 µM; AA 1 mM). The light transmission is recorded for 3 min (5 min for epinephrine), and the results expressed as percentage of maximal increase in light-transmission (LT%).

For PFD patients, the previous diagnosis was confirmed by evaluating the platelet secretion and δ-granules content. For patients on APT, a new parameter, platelet aggregation level (PAL) score, was developed in the CS-2400 analyser to monitor the effect of APT on platelet function[20,21]. The PAL score obtained using ADP as the agonist is called ADP-induced PAL (APAL), whilst the PAL score obtained using collagen is called collagen-induced PAL (CPAL). APAL and CPAL were calculated on the aggregation waves obtained using two concentrations of the considered agonists: ADP 1 and 10 µM and collagen 1 and 5 µg/mL (Revohem aggregation panel reagents).

Statistical analysis

Median and interquartile range (IQR) were used for continuous variables, and count and percentage were used to describe demographic and categorical clinical variables. Differences between groups were analysed using the unpaired non-parametric Mann-Whitney U test, while comparison between two methods in the same cohort of individuals was performed using the paired non-parametric Wilcoxon test. Threshold p-value for significance was set at 0.05. All reported p-values are two-sided. Receiver operating characteristic (ROC) curves were used to assess which parameters better discriminated the most severe from the milder defects. Areas under the ROC curves (AUC) were used as estimates of the predictive capability of the method. Correlations between IPF%, mean platelet volume (MPV), APAL and CPAL score were evaluated with the Spearman's Rho correlation coefficient test. All analyses were performed with the statistical software SPSS (release 27.0, IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, USA).

RESULTS

Study participants comprised 23 patients (6 males) with a median age of 56 years (range 20-75) and a previous diagnosis of congenital or acquired PFD, divided into

12 PSD and 11 δ-SPD (data obtained at the time of diagnosis are reported in **Table I**); 28 patients (14 males) with a median age of 66 years (range 23-87) on chronic daily treatment with APT (15 with clopidogrel 75 mg od, 9 with clopidogrel 75 mg od + ASA 100 mg od, 4 with ASA 100 mg od); and 19 HC (12 males) with a median age of 39 (range 18-59) years.

Patients with platelet function defects

Platelet aggregation performed with Chronolog and CS-2400 aggregometers showed a statistically significant reduction in the maximal LT% in the group of patients with PFD compared to HC, irrespective of the agonist used. Interestingly, the median LT% in both PFD patients and HC obtained with CS-2400 aggregometer were significantly higher than those obtained with Chronolog aggregometer (**Figure 1**).

To evaluate the ability of the LT% measured with CS-2400 to distinguish patients with a PFD from HC, a ROC curve analysis was performed. ROC curves showed that the LT% measured with CS-2400 with all agonists was an effective discriminator for distinguishing between the two groups (area under the ROC curve >0.70) (*online supplementary* **Figure S1).** Youden index analysis was used to calculate the cut-off values of the LT% with the best trade-off between sensitivity and specificity for discriminating HC (>cut-off) from PFD sufferers (<cut-off). At this point, an agreement test of HC/PFD diagnosis was made at our centre with Chronolog aggregometer and the cut-off values of the LT% measured with CS-2400, and a reasonable degree of concordance emerged, with a Cohen's k coefficient between 0.5 and 0.6 according to the used agonist.

Table I - *Median values (IQR) of percentage of light transmission, release of adenosine triphosphate (ATP) and intraplatelet granule content, serotonin (5HT) and the nucleotides adenosine diphosphate (ADP) and ATP, in platelet function defects (PFD), platelet secretion defects (PSD) and δ-storage pool disease (δ-SPD) obtained at the time of diagnosis*

	Normal values	PFD (No.=23)	PSD (No.=12)	δ-SPD (No.=11)	p-value PSD *vs* δ-SPD
Platelet aggregation (%)					
ADP (4 µM)	>58	45 (34-56)	52 (41-58)	41 (31-52)	0.276
ADP (20 µM)	>64	61 (50-76)	72 (58-79)	51 (46-63)	0.023
Collagen (2 µg/mL)	>66	72 (35-81)	81 (72-84)	25 (15-62)	0.002
U46619 (0.5 µM)	>53	51 (9-78)	74 (22-83)	4 (4-50)	0.034
U46619 (1 µM)	>65	68 (44-83)	83 (69-90)	51 (24-61)	0.005
TRAP (10 µM)	>48	20 (14-55)	27 (14-85)	17 (8-29)	0.106
Arachidonic acid (1 mM)	>62	64 (51-78)	75 (64-83)	49 (15-65)	0.003
Released ATP (nmol/10^8 platelets)					
ADP (4 µM)	0.022-0,098	0.000 (0.000-0.000)	0.000 (0.000-0.000)	0.000 (0.000-0.000)	0.186
ADP (20 µM)	0.036-0,612	0.000 (0.000-0.036)	0.033 (0.000-0.055)	0.000 (0.000-0.000)	0.030
Collagen (2 µg/mL)	0.168-0,932	0.354 (0.060-0.422)	0.402 (0.356-0.452)	0.028 (0.000-0.098)	<0.001
U46619 (0.5 µM)	0.018-1,270	0.000 (0.000-0.180)	0.142 (0.000-0.207)	0.000 (0.000-0.000)	0.008
U46619 (1 µM)	0.100-1,030	0.113 (0.000-0.242)	0.214 (0.129-0.280)	0.000 (0.000-0.086)	0.003
TRAP (10 µM)	0.012-1,074	0.000 (0.000-0.065)	0.000 (0.000-0.389)	0.000 (0.000-0.000)	0.071
Arachidonic acid (1 mM)	0.201-1,020	0.308 (0.270-0.478)	0.451 (0.295-0.559)	0.121 (0.000-0.342)	0.005
Intraplatelet granules content					
5HT (nmol/10^8 plts)	0.23-0.58	0.31 (0.16-0.42)	0.41 (0.35-0.52)	0.16 (0.12-0.18)	<0.001
ADP (nmol/10^8 plts)	1.23-3.91	1.22 (0.50-2.02)	2.03 (1.82-2.62)	0.53 (0.34-1.05)	<0.001
ATP (nmol/10^8 plts)	3.86-7.82	4.27 (3.65-6.12)	4.11 (3.98-6.22)	4.43 (3.40-5.67)	0.560
ATP/ADP	1.43-3.26	3.77 (2.08-8.98)	1.90 (1.76-2.48)	8.41 (3.91-11.76)	0.003

p-value calculated with Mann-Whitney U test. *p<0.05.

Online supplementary **Table SI** reports the results of a sub analysis conducted in the cohort of PFD patients evaluated with CS-2400. Following our diagnostic criteria, an increase in LT% below the 5th percentile of the distribution of results from HC (<48%, <69%, <88%, <88%, <76%, <28% with CS-2400, for ADP 2-4-20 µM, collagen 2 ug/mL, AA 1 mM and epinephrine 5 µM, respectively) was defined as abnormal aggregation, while an increase in LT% above the 5th percentile was defined as normal aggregation. CS-2400 identified the patients with an abnormal LT% with strong

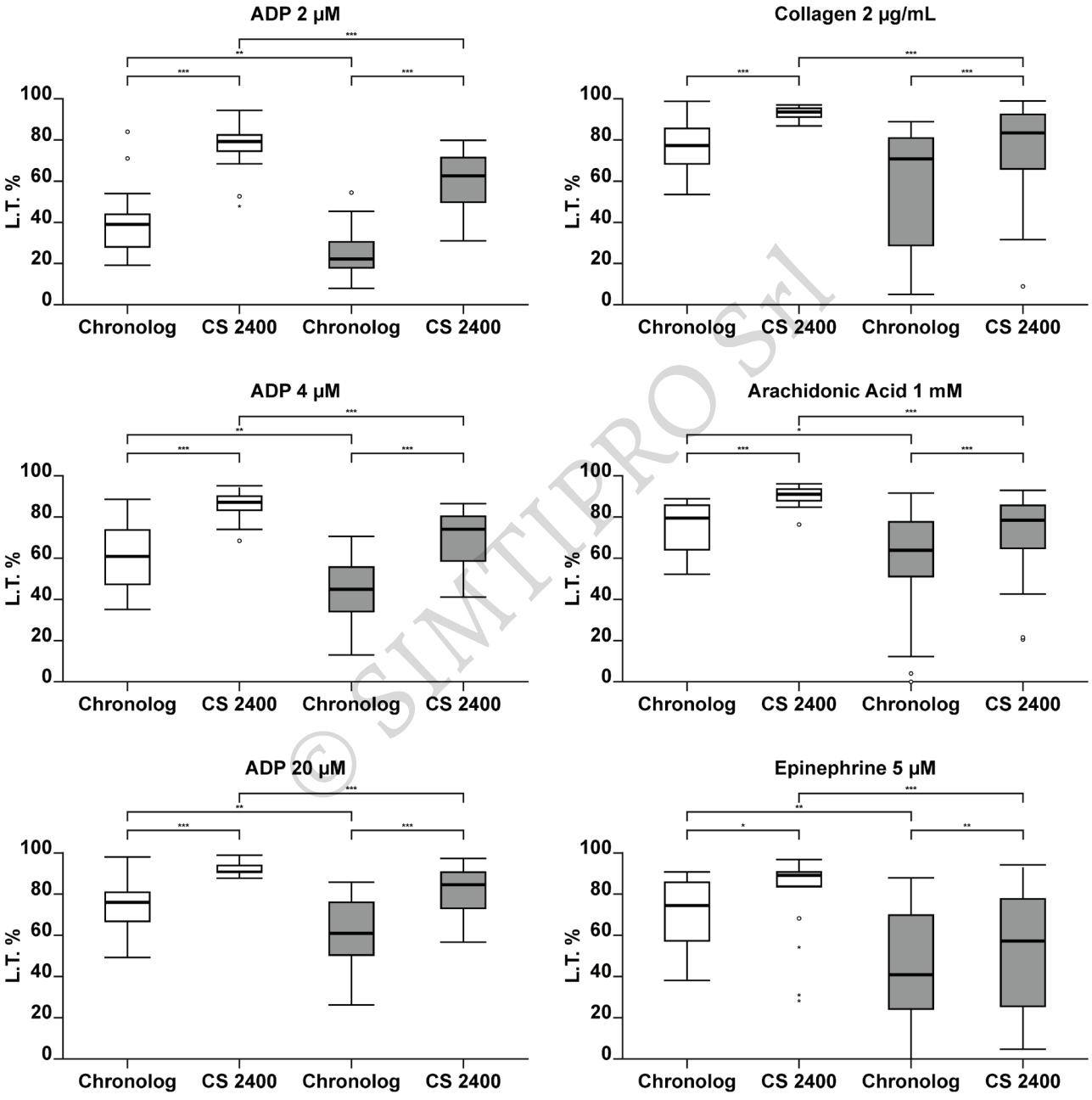

Figure 1 - Percentage of light transmission (LT%) in Chronolog aggregometer with routinely used agonists and CS-2400 aggregometer with Revohem agonists in healthy controls (white boxes) and in bleeding patients with platelet function defects (grey boxes)

p-value calculated by the unpaired non-parametric Mann-Whitney U test for differences between groups and by the paired non-parametric Wilcoxon test between two methods in the same cohort of individuals *p<0.05; **p<0.01; ***p<0.001.

agonists (ADP at the higher concentration, collagen and AA) whereas when weak agonists were considered (ADP at the two lower concentrations and epinephrine), CS-2400 classified the patients as normal, proving less effective at identifying patients with a low response to weak agonists.

Patients on antiplatelet drugs

Platelet aggregation performed with Chronolog and CS-2400 aggregometers showed a statistically significant reduction in the maximal LT% in patients on APT compared to HC with all agonists (**Figure 2**).

Subsequently, patients were divided as having achieved a complete drug response when the LT% was below the 5th percentile of the distribution in the HC (<35%, <49%, <55% for Chronolog and <73%, <89%, <84% for CS-2400, with ADP 4 µM, ADP 20 µM and AA 1 mM, respectively) and an absent response when above with all agonists. A partial response was defined as an LT% reduction below the 5th percentile with at least one agonist. An agreement test between CS-2400 and Chronolog showed a fair degree of concordance, with a Cohen's k coefficient of 0.421. More in detail, CS-2400 as compared to Chronolog identified 23 *vs* 20, 4 *vs* 3 and 1 *vs* 5 patients as having a complete, partial, or absent response, respectively (*data not shown*).

In all patients on APT, the median value (IQR) of the

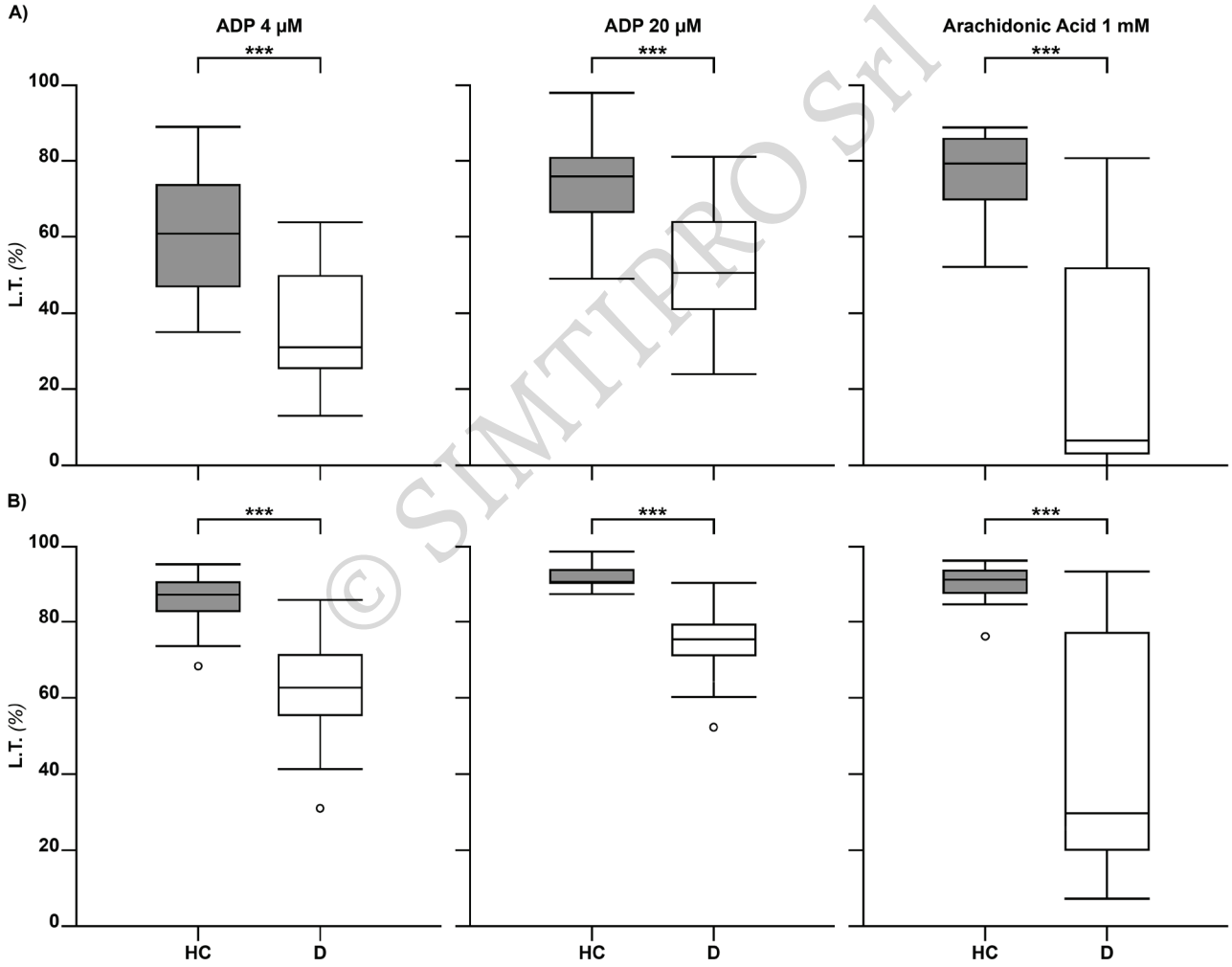

Figure 2 - Percentage of light transmission (LT%) obtained with Chronolog aggregometer with routinely used agonists (panel A) and CS-2400 aggregometer with Revohem agonists (panel B) in healthy controls (HC) and patients on treatment with antiplatelet drugs (D)

p-value calculated with Mann-Whitney U test: *** p<0.001.

APAL and CPAL score was significantly lower than in HC (6.4 [5.9-8.0] *vs* 9.7 [8.8-10.0] and 7.1 [5.7-8.5] *vs* 10.0 [10-10] respectively) (**Figure 3**, **Table II**). Median (IQR) platelet count was significantly lower in patients than in the control group (216 [191-241] *vs* 253 [231-292] × 10^3/uL; p<0.05) whereas there was no significant difference in IPF% (4.6 [3.3-6.6] *vs* 3.9 [2-5.2]) and MPV (10.8 [10.4-11.5] fL *vs* 10.8 [9.3-11.5] fL). As expected, there was a positive correlation

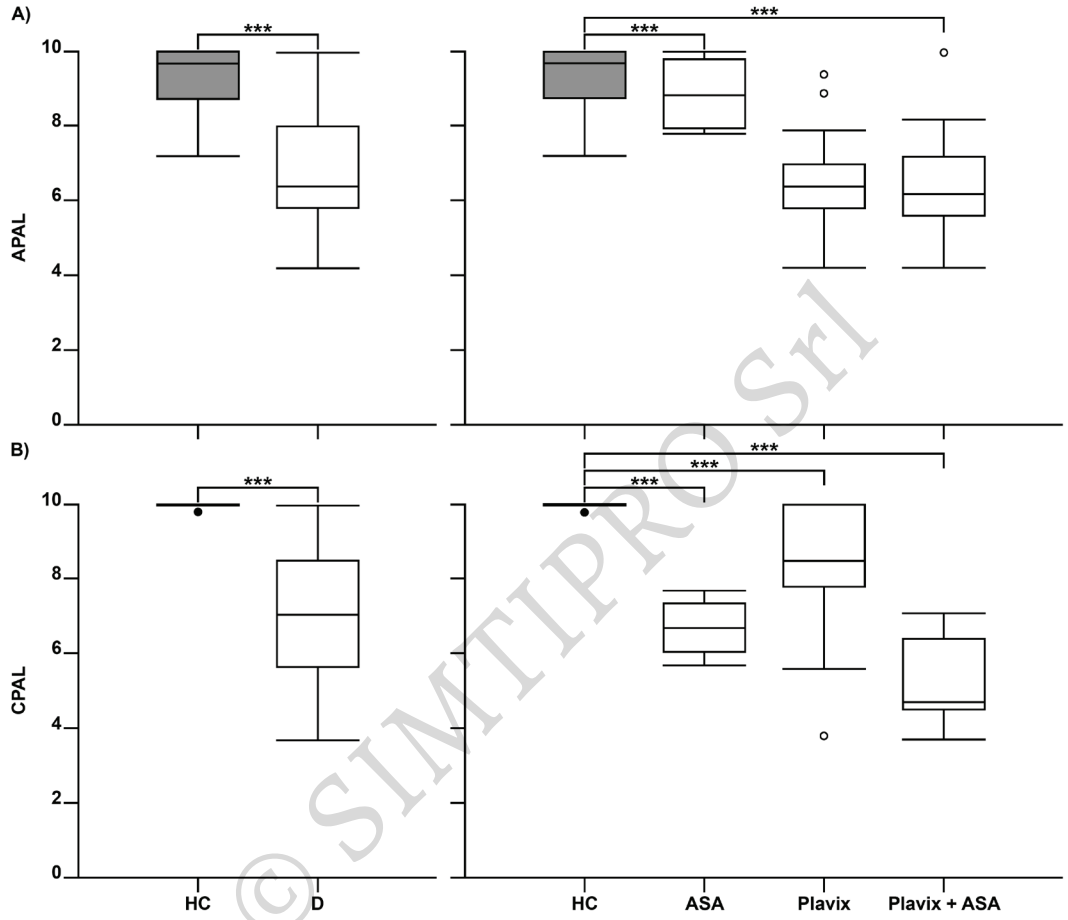

Figure 3 - APAL and CPAL scores (on the left) in healthy controls (HC) and patients on treatment with antiplatelet drugs (D) evaluated with CS-2400 aggregometer and Revohem agonists and (on the right) in HC and in patients on treatment with ASA/Plavix/Plavix + ASA
p-value calculated with Mann-Whitney U test: *** p < 0.001.

Table II - *Median (IQR) of APAL and CPAL scores in healthy controls and all patients on treatment with antiplatelet drugs, in ASA/Plavix/Plavix+ASA, evaluated with CS-2400 aggregometer and Revohem agonists*

	APAL	p-value *vs* HC	CPAL	p-value *vs* HC
HC (No.=19)	9.7 (8.8-10.0)		10.0 (10.0-10.0)	
D (No.=28)	6.4 (5.9-8.0)	<0.001	7.1 (5.7-8.5)	<0.001
ASA (No.=4)	8.9 (8.0-9.7)	0.362	6.7 (6.2-7.2)	<0.001
PLAVIX (No.=15)	6.4 (5.8-7.0)	<0.001	8.5 (7.8-10.0)	<0.001
PLAVIX + ASA (No.=9)	6.2 (5.6-7.2)	<0.001	4.7 (4.5-6.4)	<0.001

p-value calculated with Mann-Whitney U test: *** p<0.001; HC: healthy controls; D: drugs; APAL: ADP-induced PAL; PAL: platelet aggregation level.

between IPF% and MPV both in controls ($p<0.001$) and patients on APT ($p<0.05$), while no correlation was found between these parameters and APAL and CPAL scores (*data not shown*).

DISCUSSION

Patients with platelet function defects

The LTA method, and particularly its modified version, lumi-LTA, which measures platelet secretion in parallel with aggregation, represents the gold standard to measure platelet function in patients with a bleeding history and a suspected defect in platelet function, due to its high sensitivity to the less severe but most common disorders characterized by defective release in presence of normal LT%[5].

The LT% of CS-2400 proved able to distinguish between HC and the historical cohort of patients with PFD with a discreet agreement (Cohen's k coefficient 0.5-0.6) although CS-2400 failed to identify cases of mild PFD, which normally have a complete response with strong agonists but normal aggregation and no secretion with weak aggregating agents such as ADP and epinephrine.

The platelet aggregation induced by agonists is subsequently amplified by the production of TxA2 from membrane phospholipids and by the secretion of ADP from the platelet delta granules. Therefore, the platelet aggregation observed in a light transmission aggregometer is that induced directly by the agonist plus the contribution of released ADP. Strong agonists such as collagen, *in vitro* directly induce platelet aggregation together with TxA2 synthesis and ADP secretion that amplify platelet aggregation response. On the other hand, weak agonists such as ADP and epinephrine, *in vitro* directly induce platelet aggregation without secretion, which is induced by the close platelet-to-platelet contact that occurs during normal platelet aggregation in presence of healthy platelet signalling pathways[5]. Based on these results, CS-2400 LTA performed very well (with a sensibility of 100% and a specificity of 83%, with collagen being the agonist with the best performance) when identifying the more severe PFD such as δ-SPD. On the other hand, CS-2400 LTA was not so effective at identifying less severe PFD, such as a PSD.

In our historical cohort of 23 PFD patients (12 PSD and 11 δ-SPD), the CS-2400 with Revohem agonists panel was able to identify 18 patients with an LT% below the 5th percentile of the distribution of results in HC with at least one agonist, including 7 out of 12 (58%) PSD and 11 out of 11 (100%) δ-SPD previously diagnosed using the gold standard test consisting lumi-LTA together with delta granule content. CS-2400 missed the diagnosis of 5 patients with a weak PSD.

These findings are not unexpected, given that LTA sensitivity to the most common PSDs is suboptimal[26], thereby confirming the need for further testing to obtain a definitive diagnosis.

The performance of automated coagulation analyzers for platelet aggregation testing has been recently been assessed using stand-alone aggregometers in other studies, which showed a good agreement between the two methods. Two studies evaluated 39 patients with a suspected PFD and 20 patients with a wide variety of hemostatic disorders, respectively[27,28]. While the first one was conducted on suspected and not already diagnosed PFD, the second one included many different hemostatic disorders, including only 3 patients with Glanzmann thrombasthenia, but without patients with the most common PFDs. Our study, on the other hand, enrolled patients with a previously confirmed diagnosis of PFD and each patient was well characterized by means of second filter tests such as secretion and intragranular dosages for the PFD subtype, including δ-SPD and PSD. Moreover, compared to these previous published works, our own study showed not only a good agreement between the two methods, but also that CS-2400 was able to identify the most severe defects, making this method suitable for initial screening of patients with a suspected PFD.

Here, we evaluated the performance of CS-2400 in a historical cohort of already diagnosed patients. In the future, the diagnostic capacity of this analyzer in terms of sensibility and specificity needs to be evaluated in a prospective study of consecutive patients referred for a bleeding history and without a previously confirmed diagnosis.

Patients on antiplatelet drugs

Platelet function tests have also been suggested for evaluating the efficacy of treatment with antiplatelet agents in addition to the diagnosis of PFD. The most commonly used antiplatelet drugs are acetylsalicylic acid (aspirin) and the antagonists of the platelet P2Y12 receptor for adenosine diphosphate (mainly clopidogrel).

Since many reports have described the variability of the individual response to these agents[29], managing to single out those patients with a poor pharmacological response is a important clinical issue, given that it is crucial to start alternative therapeutic approaches promptly. Patients with a poor response to APT may be identified with platelet function tests, even though are many issues about this approach which still need to be resolved[29]. Although there are several point-of-care devices that specifically address ADP receptor compliance (including PFA-200 with the P2Y cartridge[30] or the VerifyNow[31]), one of the conventional methods for testing platelet responsiveness is LTA[32]. However, given that platelet aggregation increases with the agonist concentration and then stabilizes once a certain concentration is reached, a single concentration of the agonist, due to intraindividual variability, may not provide sufficient information on the treatment efficacy in the examined patient. Therefore, in order to better evaluate the effect of APT, Sysmex technology has developed a new scoring method that compares the results obtained from two different concentrations of the same agonist (high and low) resulting in an index called platelet aggregation level (PAL). PAL score has been designed for two agonists: ADP to evaluate the antiplatelet activity of P2Y12 receptor antagonists and collagen to evaluate the response to COX-1 inhibitors. These two agonists are used at low and high concentrations. The score calculated from the two ADP concentrations is termed ADP-induced PAL (APAL), and that calculated from the two collagen concentrations as collagen-induced PAL (CPAL)[15]. These scores may range from 0 to 10. Higher PAL score values indicate higher levels of platelet aggregation, reflecting a poor or absent response to APT, while lower PAL score values indicate lower levels of platelet aggregation, reflecting an appropriate response to APT.

To evaluate the usefulness of CS-2400 in monitoring the effect of APT, our study measured the LT% on CS-2400 and compared it to Chronolog in a group of patients on APT. Test agreement between the two analysers amounted to 82%. Twenty-three out of 28 patients had a concordant drug response with the two methods, and 5 had a reduced response with CS-2400, indicative of greater APT efficacy. More specifically, CS-2400 identified 27 patients as effectively anti-aggregated (23 with complete and 4 with partial response) compared to the 23 identified by Chronolog, therefore overestimating the response to the antiplatelet agent. The sample size was too low to reach any firm conclusions, but these results highlight that LT% with CS-2400 is not an optimal tool for initial screening, due to its high sensitivity to antiplatelet agents. On the other hand, by comparing two concentrations of the same agonist, the PAL scores reduce the variability in the same subject and are more reliable as measurements of the efficacy of antiplatelet agents compared to LT% alone.

As expected, the PAL scores varied in a drug-dependent manner, reflecting their mechanism of action, with the APAL score being lower in patients on P2Y12 antagonists and CPAL score lower in patients on COX-1 inhibitors. Furthermore, both median APAL and CPAL scores were lower in dual compared to single drug therapy, confirming *in vitro* studies[14].

Until now, no clinical studies have established the cut-off values of anti-aggregation that correlate with thrombotic event relapse while on treatment, and prospective studies are needed to address this issue. Moreover, there are still no cut-off values for the PAL scores which can distinguish between a good or bad platelet response to APT. However, the present study has shown the potential of these scores: further investigations with a higher number of patients are now necessary, so as to confirm their significance and usefulness.

CONCLUSIONS

Our study shows that the automatic analyzer CS-2400 compares favourably with a stand-alone device, Chronolog-LTA, in both PFD detection and APT response evaluation.

CS-2400 was fairly good at distinguishing HC from patients with PFD and was good at distinguishing patients with PSD from patients with δ-SPD. Moreover, the newly developed PAL scores showed great promise in their capacity for evaluating residual platelet activity in patients on APT.

In conclusion, thanks to its easy handling and its ability to perform routine coagulation together with platelet aggregation tests, we believe that CS-2400 could become useful for initial screening in those patients with a bleeding diathesis and suspected defect in platelet function, which needs to be confirmed with more specific tests. Moreover, in combination with PAL scores, it could also be used to evaluate platelet response to APT.

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AUTHORS' CONTRIBUTIONS

AL and FP designed the study. MC, AA, LP and SLM collected data. LD and SLM analysed the data. AL and MC interpreted data and wrote the manuscript. All the Authors helped to revise and approve the final version of the manuscript.

DISCLOSURE OF CONFLICTS OF INTEREST

FP has received honoraria for participating as a guest speaker in education meetings organized by Grifols and Roche, and she is a member of the Scientific Advisory Boards of Biomarin, Roche, Sanofi, Sobi, and Takeda. The other Authors have no conflicts of interest to disclose.

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