Flow-chamber device (T-TAS) to diagnose patients suspected of platelet function defects

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Background - Patients suspected of platelet function defects represent a diagnostic challenge for the clinical laboratory, mainly due to the complexity and poor standardization of screening methods. We compared a new flow-based chip-equipped point-of-care (T-TAS) device with lumi-aggregometry and other specific tests.

Materials and methods - The study included 96 patients suspected of platelet function defects and 26 patients referred to hospital for an evaluation of residual platelet function while on antiplatelet therapy.

Results - Forty-eight of 96 patients displayed abnormal platelet function by lumi-aggregometry, and 10 of them had defective granule content and were classified as δ-storage pool disease (δ-SPD). T-TAS compared favorably with lumi-aggregometry in detecting the most severe forms of platelet function defects (i.e., δ-SPD) [test agreement (lumi-light transmission aggregometry [lumi-LTA] vs T-TAS) for the δ-SPD subgroup was 80% and K CHOEN 0.695. T-TAS was less sensitive to milder platelet function defects (i.e., primary secretion defects [PSD]). Concerning patients on antiplatelets, test agreement (lumi-LTA vs T-TAS) in detecting patients who were responders to this therapy was 54%; K CHOEN 0.150.

Discussion - The results indicate that T-TAS can detect the more severe forms of platelet function defects such as δ-SPD. There is limited agreement of T-TAS with lumi-aggregometry in identifying responders to antiplatelets. However, this poor agreement is commonly shared by lumi-aggregometry and other devices owing to the lack of test specificity and of prospective data from clinical trials linking platelet function with therapeutic efficacy.

Keywords: *primary hemostasis, platelet aggregation, coagulation, antiplatelet drugs, laboratory investigation.*

INTRODUCTION

Hemostasis is a complex tightly regulated cellular/humoral mechanism which serves to maintain blood flow in the normal circulation and to stop bleeding at the site of vessel wall injury. Among the cellular components, a crucial role is played by platelet-vessel wall interactions, collectively known as primary hemostasis, whereby platelet adherence to

the subendothelial matrix at the site of vessel wall injury is followed by aggregation. Adhesion and aggregation mediated by von Willebrand factor (VWF) and either VWF or fibrinogen lead to thromboxane (TXA2) and ADP formation. Both are potent platelet pro-aggregatory substances which favor release from dense and alpha platelet granules of stored substances that stabilize aggregation. Besides primary hemostasis, platelets play a crucial role in secondary hemostasis or coagulation by exposing negatively charged phospholipids on their surface that act as cellular receptors for vitamin K-dependent coagulation factors, thus localizing thrombin generation and fibrin formation at the site of the vessel wall injury.

Congenital and acquired abnormalities of platelet function are associated with bleeding. Hence, platelet function tests are frequently included in the laboratory workup of patients experiencing bleeding episodes. However, while blood coagulation can be investigated even in less specialized laboratories, primary hemostasis testing requires a level of expertise that is not usually available in non-specialized laboratories. Platelet function tests are also prescribed to identify platelet hyperactivity as a possible predictor of thrombosis recurrence in patients who, owing to past episodes of ischemic or thrombotic events of the coronary or cerebral vascular districts, are on antiplatelet medication. Therefore, investigation of primary hemostasis is an unmet need and new simple methods that could be used in clinical laboratories need to be found and evaluated. This article aims to compare the laboratory results obtained in a relatively large number of well-characterized patients who were referred to hospital either because of suspected platelet function defects or for residual platelet activity testing while on antiplatelet therapy. Laboratory tests used for the comparative evaluation were the lumi-light transmission aggregometry (lumi-LTA) and the Total Thrombus Analysis System (T-TAS) that was designed to investigate overall primary hemostasis function.

MATERIALS AND METHODS

Study design and patient population

The primary objective of the study was the evaluation of the efficacy of T-TAS to detect congenital or acquired disorders of platelet function in patients referred to our hospital because of unexplained bleeding. We investigated a group of 96 consecutive outpatients (27 men, 69 women, median [min-max] age 40 [7-78] years) over the period October 2019-July 2021. They presented a mild to moderate bleeding diathesis of variable severity, characterized by mucocutaneous bleeding, menorrhagia, and/or excessive post-surgical and post-traumatic blood loss. The study cohort all had the following laboratory parameters within normal limits: platelet count and size, prothrombin time (PT) and activated partial thromboplastin time (APTT), VWF (antigen and ristocetin co-factor activity) and factor (F)VIII activity.

A secondary objective was to evaluate the ability of T-TAS to monitor the effects of drugs in patients with coronary artery disease on chronic treatment with antiplatelet drugs (No.=16: clopidogrel 75 mg; No.=6: clopidogrel 75 mg+ASA 100 mg No.=4: ASA 100 mg). Fifty healthy subjects with no personal or family history of hemorrhage/thrombosis, who over the previous 10 days had not taken antithrombotic medication or any drug known to interfere with platelet function were investigated as controls. Results from controls were also used to determine the limits of the reference range for T-TAS; limits of the reference range for lumi-LTA were those historically determined in the laboratory.

The study was approved by the institutional review board and all study participants gave informed consent to donate small amounts of blood for the study in addition to those needed for their routine investigation.

Blood sampling

Blood was drawn with plastic syringes. The first three mL were collected into K-EDTA tubes (Sarstedt, Verona, Italy) for blood count, the next 30mL were collected in non-evacuated home-made prepared tubes containing trisodium citrate (109 mM) for testing with lumi-LTA. To analyze hemostatic plug formation (T-TAS), blood was anticoagulated with benzilsulfonil-D-Arg-Pro-4-amidinobenzilamide[1,2] provided by the manufacturer.

Platelet aggregation and secretion

Platelet function disorders (PFD) were assessed in platelet-rich plasma (PRP) by lumi-LTA (Chronolog, Mascia-Brunelli, Milan, Italy). Since the pattern and extent of aggregation abnormalities vary among individuals, it is essential to evaluate secretion and aggregation to determine whether PFD are present[3]. PRP or platelet-poor plasma (PPP) were prepared by centrifugation

of citrated blood at 200 or at 1,400 *g* for 10 or 15 minutes (min), respectively. The original PRP platelet counts were used to measure platelet function[4]. After incubation with luciferin/luciferase to evaluate ATP secretion, the following agonists were used: adenosine-diphosphate (ADP) 4 μM, collagen 2 μg/mL, U46619 (TXA2 analog) 1 μM, 14-residue-thrombin-receptor-activator peptide (TRAP-14) 10 μM and arachidonic acid (AA) 1 mM. Results were expressed as maximal percentage increase in light-transmission (the smaller the percentage of light-transmission, the more likely the impairment of the platelet function). A standard curve using ATP doses added *in vitro* to PPP was used to quantify secretion expressed as nmol/10^8 platelets.

To determine if the platelet function abnormalities were primary defects of platelet biogenesis delta storage pool diseases (δ-SPD) or signaling pathway disorders leading to secretion defects of platelet granule (PSD), we also measured substances contained in delta granules. Total platelet ADP and ATP were measured with luminometer (LKB 1250, Bio-Orbit Oy, Turku, Finland) and the firefly luciferin/luciferase method[5]. Platelet serotonin was measured by the o-phthaldialdehyde method[6].

Flow-based thrombus formation

To analyze flow-based thrombus formation, the modified T-TAS (Zacros, Fujimori Kogyo, Tokyo, Japan) was used. T-TAS is a flow-chamber device that analyzes platelet plug formation by type 1 collagen-coated microchips. Blood was perfused into the 26-capillary path of the microchips with a precision pump and parameters were automatically recorded. Briefly, whole blood (350 μL) was applied to the platelet microchip at a flow rate of 24 μL•min^{-1}, corresponding to 2000s^{-1} wall shear rate (equivalent to an approximate arteriolar shear stress). The flow pressure curves were assessed for 10 min and platelet plug formation was evaluated using the following parameters: (i) occlusion start time (OST), defined as the time (min) needed to reach the occlusion start-pressure of 10 kPa; (ii) area under the flow pressure curve (AUC) below 60 kPa for 10 min, which quantifies plug stability (min•kPa); (iii) occlusion time (OT), which defines the time (min) at which 60 kPa fixed occlusion pressure is reached. Prolonged OST, OT and low AUC are considered indexes of platelet function defects.

Platelet aggregation in patients on antiplatelet drugs

Antiplatelet therapy was assessed by lumi-LTA and results were expressed as maximal increase in light transmission when tested with the agonists ADP (4 and 20 μM) and AA (1 mM). A maximal percentage increase in light transmission smaller than the 5th percentile of the distribution of results from controls (<35%, <49%, <55% with ADP-4 μM, ADP-20 μM, AA-1 mM) was taken as an indication of poor residual platelet reactivity and therefore as good response to antiplatelet therapy; high residual platelet reactivity was considered poor or absent response.

Statistical analysis

Quantitative variables for parameters of lumi-LTA and T-TAS are reported as median (min-max). Differences between groups were analyzed by the non-parametric Mann-Whitney U test. Receiver operating characteristic (ROC) curves were used to assess which of the T-TAS parameters better discriminated the more severe from milder defects. Areas under ROC curves (AUC) were used to estimate the predictive value of the method. Two-sided p <0.05 was considered statistically significant. Analyses were performed by SPSS, version 27.0, and the statistical software R, release 4.0.0.

RESULTS

Platelet aggregation and secretion in bleeding patients

Laboratory data on PFD were available for 96 patients with a bleeding diathesis and suspected PFD were initially evaluated by lumi-LTA. The values of maximal percent light transmission are shown in **Figure 1**. Forty-eight of 96 patients showed normal aggregation and are identified hereafter as patients with a non-confirmed diagnosis (PNC). In contrast, the remaining 48 showed significantly (p<0.05 or p<0.001) reduced percent light transmission (irrespective of the agonist used) and were, therefore, diagnosed as having PFD owing to impaired aggregation, such as an absent secondary wave, decreased slope, or reversible aggregation. These abnormalities resulted primarily from impaired ATP release from delta granules, as shown by the finding that ATP secretion expressed as nmol/10^8 platelets was significantly reduced with all agonists for PFD compared to PNC (p<0.01 or p<0.001) (**Table I**). To determine whether the defective secretion was due to an abnormal platelet biogenesis

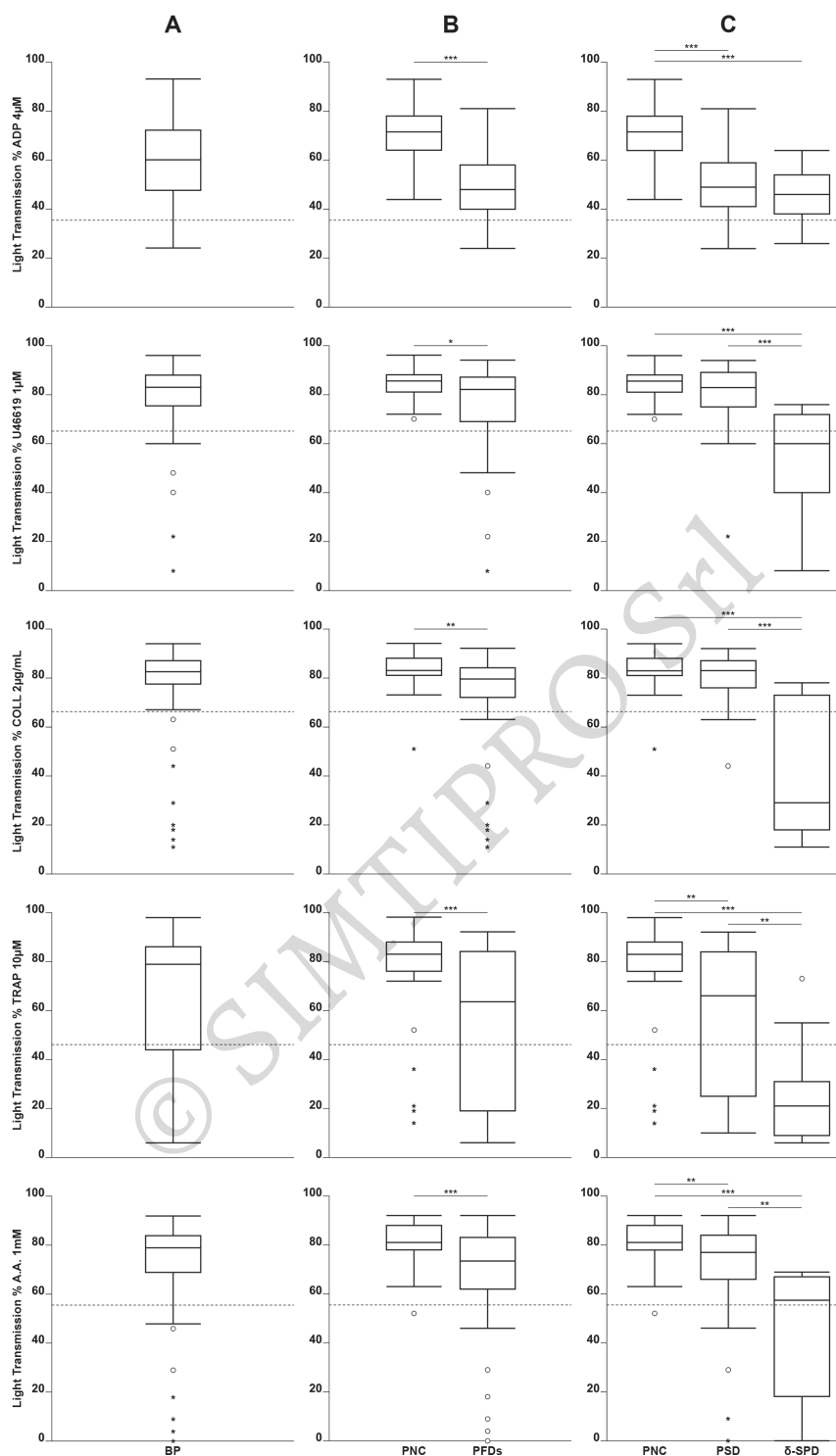**Figure 1 - Distribution of percent light transmission in patients with normal coagulation and a history of bleeding**
(**A**) BP: all 96 bleeding patients (irrespective of the final diagnosis based on lumi-light transmission aggregometry [lumi-LTA]). (**B**) PNC: 48 bleeding patients without confirmed platelet defect based on lumi-LTA; PFD: 48 bleeding patients with confirmed platelet defect based on lumi-LTA. (**C**) PNC: 48 bleeding patients without confirmed platelet defect based on lumi-LTA; PSD: 38 bleeding patients diagnosed as having platelet secretion defect; δ-SPD: 10 bleeding patients diagnosed as having δ-storage pool disease. Dashed line indicates 5th percentile of distribution of healthy controls. *p<0.05, **p<0.01, ***p<0.001.

Table I - *Median (min-max) of released ATP (nmol/10⁸ platelets) for patients with normal coagulation and a history of bleeding, when induced by ADP, COLL, U46619, TRAP, AA*

	PNC (No.=48)	PFDs (No.=48)	p-value PNC *vs* PFDs	PSD (No.=38)	p-value PNC *vs* PSD	δ-SPD (No.=10)	p-value PNC *vs* δ-SPD	p-value PSD *vs* δ-SPD
ADP 4µM (0.022-0.982 nmol/10⁸PLT)*	0.081 (0-0.561)	0.000 (0-0.209)	<0.001	0 (0-0.116)	<0.001	0 (0-0.209)	<0.001	n.s.
COLL 2µg/mL (0.168-0.932 nmol/10⁸PLT)*	0.504 (0.209-0.950)	0.377 (0-0.869)	<0.01	0.427 (0.196-0.869)	<0.05	0.072 (0-0.406)	<0.001	<0.001
U46619 1µM (0.100-1.030 nmol/10⁸PLT)*	0.315 (0.097-0.673)	0.197 (0-0.903)	<0.001	0.208 (0-0.903)	<0.01	0.043 (0-0.231)	<0.001	<0.001
TRAP 10µM (0.012 -1.074 nmol/10⁸PLT)*	0.472 (0-1.032)	0.176 (0-0.785)	<0.001	0.249 (0-0.785)	<0.001	0.000 (0-0.289)	<0.001	<0.01
AA 1mM (0.201 -1.020 nmol/10⁸PLT)*	0.540 (0.140-0.920)	0.409 (0.140-1.061)	<0.01	0.427 (0-1.061)	<0.05	0.127 (0-0.580)	<0.001	<0.05

*Laboratory reference range. ADP: adenosine diphosphate (ADP); COLL: Horm Collagen; U46619: Thromboxane A2 analog; TRAP: 14-residue thrombin receptor activator peptide; AA: arachidonic acid; PNC: bleeding patients without confirmed platelet defect based on lumi-light transmission aggregometry (lumi-LTA); PFD: bleeding patients with confirmed platelet function defect; PSD: bleeding patients diagnosed as having platelet secretion defect; δ-SPD: bleeding patients diagnosed as having δ-storage pool disease.

or to signaling pathway disorders, we measured delta granule contents. Median concentrations of delta granule content (serotonin (0.19 *vs* 0.38nmol/10⁸platelets); ADP (0.87 *vs* 2.01nmol/10⁸ platelets) were lower in 10 of 48 patients with PFD, so that these patients were classified as δ-SPD. In contrast, the 38 patients with a defective aggregation/secretion with normal platelet delta granule contents were classified as having PSD (**Table II**).

Flow-based thrombus formation

Results for the T-TAS parameters are shown in **Figure 2**. There was no significant difference in median T-TAS parameters between all investigated patients and controls (i.e., AUC [378 *vs* 379]; OST [2.2 *vs* 2.0 min] or OT [4.9 *vs* 4.9 min]).

Forty-eight patients diagnosed with lumi-LTA as having PFD showed median T-TAS parameters that differed slightly to the median of the 48 PNC (i.e., AUC [358 *vs* 388]; OST [2.2 *vs* 2.1 min] or OT [5.3 *vs* 4.6 min]). The analysis of the δ-SPD subgroup showed markedly reduced median AUC (22 *vs* 377) and markedly increased OST (9.3 *vs* 2.1 min) or OT (11 *vs* 5 min) when compared to the PSD subgroup, who, in contrast, presented with similar median AUC (377 *vs* 358); OST (2.1 *vs* 2.2 min) or OT (4.9 *vs* 5.3 min) to the 48 patients identified as PFD. The PSD cases had T-TAS median parameters similar to those of the 48 PNC (i.e., AUC [377 *vs* 388]; OST [2.1 *vs* 2.1 min] or OT [4.9 *vs* 4.6 min]). Test agreement (lumi-LTA *vs* T-TAS) for the δ-SPD cases, calculated on results from the T-TAS parameter OT>10 min was 80% (8/10); K CHOEN 0.695 (*Online Supplementary* **Table SI**).

Table II - *Median (min-max) delta granules contents for 5HT, ADP and ATP for patients with normal coagulation and previous history of bleeding*

	PNC (No.=48)	PFDs (No.=48)	p-value PNC *vs* PFDs	PSD (No.=38)	p-value PNC *vs* PSD	δ-SPD (No.=10)	p-value PNC *vs* δ-SPD	p-value PSD *vs* δ-SPD
5HT (0.23-0.58 nmol/10⁸PLT)*	0.47 (0.01-0.91)	0.38 (0.02-1.03)	<0.05	0.40 (0.04-1.03)	n.s.	0.17 (0.02-0.42)	<0.01	<0.001
ATP (3.86-7.82 nmol/10⁸PLT)*	5.31 (1.71-8.02)	4.77 (2.53-10.62)	n.s.	4.91 (3.85-10.62)	n.s.	4.43 (2.53-7.52)	n.s.	n.s.
ADP (1.23-3.91 nmol/10⁸PLT)*	2.50 (1.50-3.49)	2.01 (0.15-3.16)	<0.05	2.19 (1.32-3.16)	n.s.	0.87 (0.15- 1.22)	<0.001	<0.001
Ratio ATP/ADP (1.43 -3.26)*	2.31 (1.63-3.21)	2.84 (1.41-24.60)	n.s.	2.36 (1.41-6.56)	n.s.	7.16 (3.54-24.60)	<0.001	<0.001

*Laboratory reference range. 5HT: serotonin; ADP: adenosine diphosphate; ATP: adenosine triphosphate; PNC: bleeding patients without confirmed platelet defect based on **lumi-light transmission aggregometry (lumi-LTA)**; PFD: bleeding patients with confirmed platelet function defect; PSD: bleeding patients diagnosed as having platelet secretion defect; δ-SPD: bleeding patients diagnosed as having δ-storage pool disease.
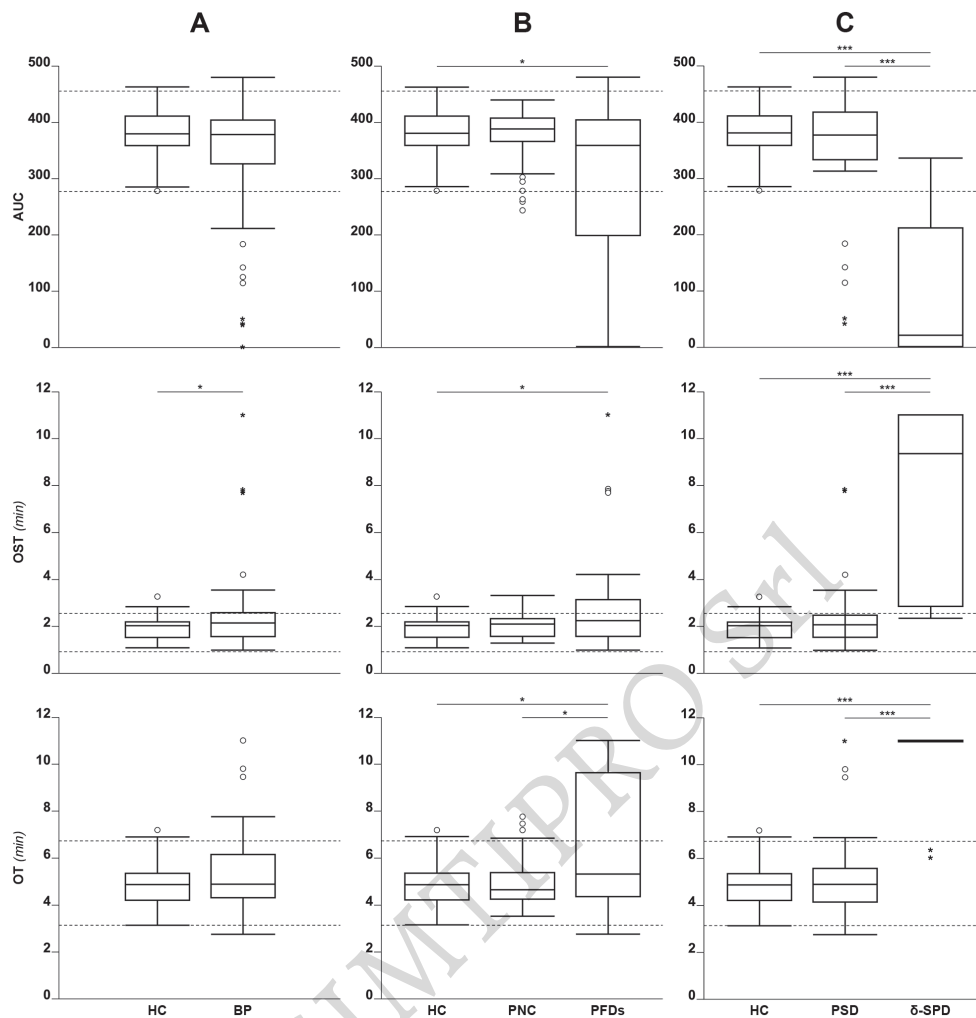
Figure 2 - Distribution of Total Thrombus Analysis System (T-TAS) results for patients with normal coagulation and a history of bleeding

(**A**) HC: 50 healthy controls; BP: all 96 bleeding patients (irrespective of the final diagnosis based on lumi-light transmission aggregometry [lumi-LTA]). (**B**) HC: 50 healthy controls; PNC: 48 bleeding patients without confirmed platelet defect based on lumi-LTA; PFD: 48 bleeding patients with confirmed platelet defect based on lumi-LTA. (**C**) HC: 50 healthy controls; PSD: 38 bleeding patients diagnosed as having platelet secretion defect; δ-SPD: 10 bleeding patients diagnosed as having δ-storage pool disease. Dashed lines indicate reference range (2.5th - 97.5th percentile of the distribution of HC). *p<0.05, **p<0.01, ***p<0.001.

To see whether any of the cut-off value for the T-TAS parameters were potentially able to discriminate between the forementioned patient subgroups, ROC curve analyses were performed. Each of the T-TAS parameters (AUC, OST, OT) had a favorable capacity (i.e., area under the ROC curve >0.90) to differentiate between the 38 patients diagnosed as PSD and the 10 patients with the most severe platelet function defects diagnosed as δ-SPD (**Figure 3**). The diagnostic efficacy for any of the T-TAS parameters

displayed no acceptable discriminatory capacity in distinguishing between the 48 patients with normal platelet function and the 48 patients diagnosed as PFD (i.e., area under the ROC curve <0.70) (*data not shown*).

Platelet reactivity in patients on antiplatelet drugs

Results obtained for lumi-LTA or T-TAS in patients on antiplatelet drugs are shown in **Figure 4**. Patients were considered as having achieved a full antiplatelet response when results for lumi-LTA measured in the presence of the
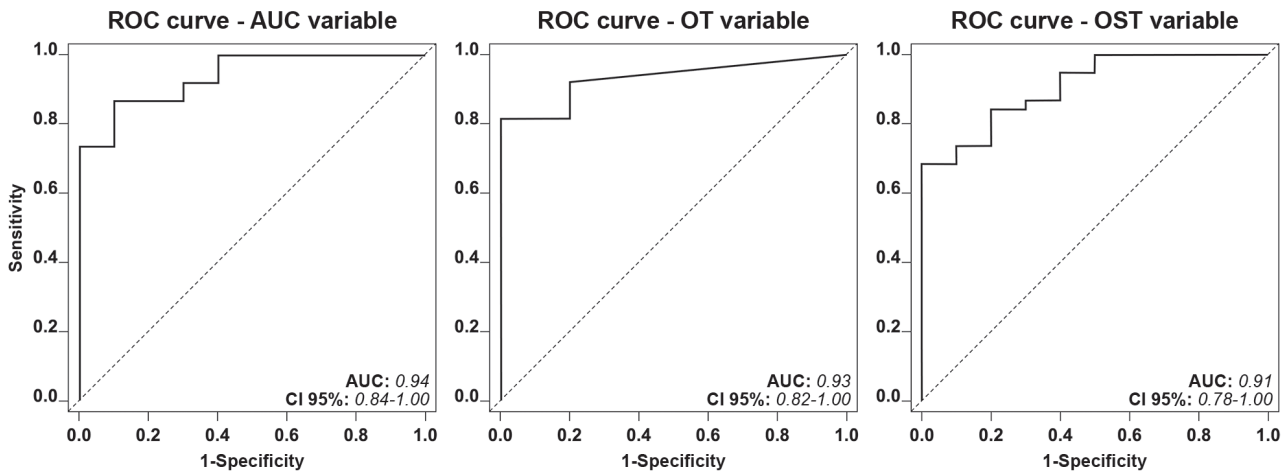
Figure 3 - **Receiver operating characteristic (ROC) curve showing the predictive ability of the full logistic model containing Total Thrombus Analysis System (T-TAS) parameters Area under Curve (AUC), occlusion time (OT), and occlusion start time (OST) for the discrimination of bleeding patients diagnosed as having δ-storage pool disease (δ-SPD) or bleeding patients diagnosed as having platelet secretion defect (PSD)**
The dashed diagonal line represents the situation of predictive uncertainty (AUC=0.50).
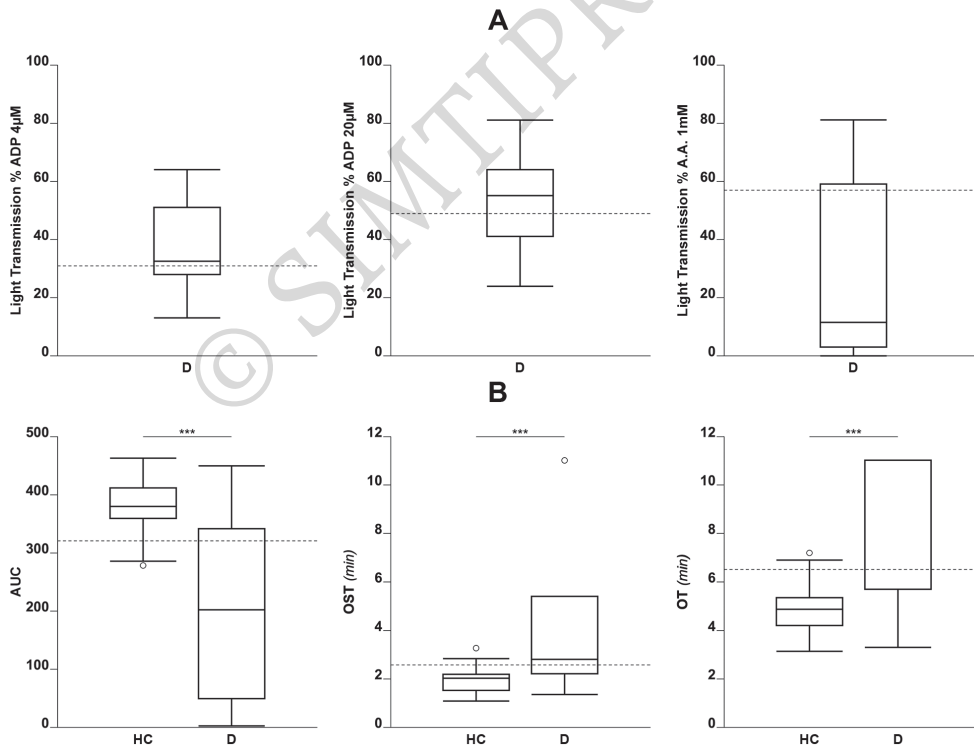
Figure 4 - Distribution of percent light transmission in patients on treatment with antiplatelet drugs when tested by lumi-light transmission aggregometry (lumi-LTA; ADP4 20µM, arachidonic acid 1mM) or by Total Thrombus Analysis System (T-TAS)
(**A**) Percent light transmission (D) in all patients on antiplatelet drugs. Dashed lines indicate 5th percentile of the distribution of healthy controls (HC). (**B**) T-TAS parameters. AUC: Area Under Curve; OST: occlusion start time; OT: occlusion time. Dashed lines indicate 5th and 95th percentile of the distribution of HC, respectively. *p<0.05, **p<0.01, ***p<0.001.

agonists were below the 5th percentile of the distribution of healthy controls (<35%, <49%, <55% with ADP 4 μM, ADP 20 μM, AA 1 mM, respectively); those above the 5th percentile were considered non-responders. Drug response when evaluated for T-TAS was based on the analysis of OST, OT or AUC taken as indexes of platelet response. Specifically, patients on treatment who had OST or OT values higher than 95th and AUC smaller than 5th percentiles of the distribution of controls were considered to have achieved complete antiplatelet response and those with OST or OT values below 95th and AUC values higher than 5th percentiles were considered non-responders. Of the 26 patients on antiplatelet therapy (16 clopidogrel, 4 ASA, 6 clopidogrel+ASA), the lumi-LTA identified 15 with complete response, 6 with no response, and 5 with partial response to therapy. T-TAS identified 17 patients with complete response, no patient with a partial response, and 9 with no response. Test agreement (lumi-LTA *vs* T-TAS) was 54% (14/26); K CHOEN 0.150 (*Online Supplementary Table SII*).

DISCUSSION

Patients with bleeding diathesis

Diagnosis of platelet function defects (PFD) requires accurate laboratory testing and many *in vitro* tests have been developed for this purpose. Among them, light transmission aggregometry (LTA) is still considered to be the most useful to screen patients suspected of having PFD. However, LTA is time consuming, technically demanding, and subject to many preanalytical and analytical variables. The modified LTA, lumi-LTA, is more sensitive to the less severe but more common platelet defects because, in parallel with platelet aggregation, it also measures platelet secretion. To help standardize aggregometry procedures across laboratories, the relevant Subcommittee of the International Society on Thrombosis and Haemostasis has issued guidelines to promote standardization[7]. However, despite these efforts, LTA and/or lumi-LTA results are not yet comparable across laboratories, suggesting that these tests should be run in specialized laboratories with high levels of expertise to perform testing and interpret results[8]. In addition, consideration should be made of the fact that aggregometry takes places in non-physiological conditions. For example, during testing, platelets in PRP or whole blood are exposed to shear forces that differ from those under *in vivo* conditions. Appropriate evaluation of platelet function would require laboratory investigation under conditions mimicking as closely as possible those *in vivo*, including blood flow and other blood cells that play crucial roles in platelet adhesion/aggregation. In this respect, flow chamber-based systems using whole blood have the potential to significantly improve the elucidation of platelet-related hemostatic mechanisms. However, these systems require special equipment and considerable technical expertise, which are often not available in non-specialized clinical laboratories[9,10]. Recently, the Total Thrombus System (T-TAS), a micro-automated system equipped with a disposable microchip flow-chamber has been made available. The device was designed to investigate platelet function in whole blood flowing through collagen-coated capillaries. While blood is flowing, platelets gradually adhere and aggregate to the surface of the capillary channels to form plugs. The system works with specimens under controlled flow and handling is straight forward, offering a system that could be useful to evaluate primary hemostasis[11,12].

In this study, we chose to compare the diagnosis obtained using standard tests (lumi-LTA, and platelet granule content) with T-TAS in relatively large numbers of patients referred to hospital for investigation of a bleeding tendency. At the time of referral, they were suspected of having defective primary hemostasis owing to a previous history of bleeding with normal platelet count/size and traditional hemostasis tests (PT, APTT, VWF antigen and VWF ristocetin co-factor activity), and after exclusion of rare hemostasis abnormalities (i.e., FXIII or plasmin inhibitor deficiencies) and/or dysfibrinogenemia. Because patients suspected of having platelet function defects still represent a formidable diagnostic challenge for the non-specialized clinical laboratory, providing a comparative evaluation of the results for the new relatively simple T-TAS device *vs* more complex tests (i.e., lumi-LTA and granule content) might improve the diagnostic workup of platelet defects.

Based on the classification established by lumi-LTA and measurement of granule content, we found that T-TAS was able to identify the patients with δ-SPD, who are among the most severe forms of platelet function defects, whereas those with milder forms such as PSD were undetectable. Our study, performed

on a consecutive population of patients with previous bleeding, confirms the results of previous studies on a characterized δ-SPD family[13]. The reasons for the failure of T-TAS to detect patients with relatively mild platelet defects is still not known. As mentioned above, T-TAS works under flow, and if one considers that flow conditions are among the most important determinants in eliciting platelet adhesion/aggregation, it is possible that modification of the flow conditions may increase the diagnostic efficacy of T-TAS towards those mild forms of platelet defects. However, the fact that T-TAS was unaffected by milder platelet defects is not surprising. A comprehensive review of the challenges faced during the laboratory investigation of patients with inherited bleeding disorders highlighted the between-assay disagreement observed for results of platelet function[14].

Platelet reactivity in patients on antiplatelet drugs

Aspirin has been historically used to prevent ischemic cardiovascular events[15,16]. However, over the last decades, new classes of antiplatelet drugs have become available including clopidogrel, prasugrel and ticagrelor[17]. These drugs have different biochemical/clinical characteristics that make them suitable for personalized treatment, for example, when some patients show drug resistance[18,19]. However, in spite of the number of devices that have been developed and the number of evaluation studies carried out, there is still no consensus on the best device to be used and/or the optimal effective/safe residual platelet activity to be reached and maintained in patients on treatment[18,20]. The present study aimed to evaluate T-TAS in comparison with LTA for a small group of patients on chronic antiplatelet drugs. Taken together, test agreement between T-TAS *vs* LTA was 54% (14/26); K CHOEN 0.150. These results suggest that T-TAS is less effective than lumi-LTA in assessing patients' response to antiplatelet drugs. A previous study on T-TAS showed that the device has a relatively good capability of detecting drug response in patients on double antiplatelet drugs (i.e., ASA + clopidogrel) when compared with patients on single therapy (i.e., ASA)[21]. The post-hoc analysis in our cohort confirmed those results. The median (min-max) T-TAS AUC of patients on double therapy (i.e., 29[3-265]) was smaller than controls and patients on single therapy (i.e., ASA 320[107-389] or clopidogrel 240[3-449]). However, the sample size was relatively small and, therefore, no conclusions can be drawn from the above results.

Some limitations of the study should be recognized. We did not apply additional laboratory tools that can be used to detect and characterize platelet defects (i.e., genotyping by means of NGS, or exome analysis). However, recent articles in the literature argue that NGS-based diagnostic strategies for causal gene identification in such a heterogeneous clinical/laboratory phenotype as primary PFD may be ineffective[22], and phenotyping analyses are ultimately needed to diagnose platelet defects. Furthermore, we did not compare T-TAS and lumi-LTA in our patient population with other devices designed to assess platelets functional defects and/or response to antiplatelet drugs (i.e., PFA-100® [Siemens Healthcare, Erlangen, Germany] Verify-Now® [Werfen, Barcelona, Spain], Multiplate® [Roche Diagnostics, Indianapolis, IN, USA], and others). However, there are so many devices available that a face-to-face comparison would have been prohibitive. In spite of this, we believe that the reported results and conclusions may be of value to non-specialized clinical laboratories engaged in the daily workup of patients suspected of platelet function defects.

CONCLUSIONS

This study reports results on an investigation of platelet function in patients with normal coagulation but a bleeding history. The study also reports on a group of patients referred to the laboratory for assessment of residual platelet hyperactivity while on chronic antiplatelet therapy.

Patients were tested using the current diagnostic test (i.e., lumi-LTA and granule content) and the relatively new flow-based chip-equipped point of care device T-TAS. Results showed that T-TAS compared favorably with lumi-LTA and granule content tests in detecting the most severe forms of platelet function defects (i.e., δ-SPD), whereas it showed poor sensitivity to those milder defects (i.e., PSD). These results are not unexpected because they reflect the complexity of the mechanisms of primary hemostasis coupled with the multifaceted role played by platelets, about which much is still not known. Its relatively simplicity in handling specimens, recording relevant parameters, and interpreting results makes the T-TAS an addition to aggregometry and a useful device for laboratory workup to investigate platelet function defects.

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