

EVALUATION OF PLATELET AGGREGATION PARAMETERS IN RESPONSE TO ACETYLSALICYLIC ACID AND THROMBOTIC HISTORY USING LIGHT TRANSMISSION AGGREGOMETRY

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Abstract. This study aimed to evaluate platelets aggregation curve (obtained using Light Transmission Aggregometry, LTA) by computing parameters (lag phase time, slope, amplitude, area under the curve, and % deaggregation) in healthy individuals before and after a low dose acetylsalicylic acid (ASA) intake and in thrombotic patients on chronic ASA therapy. ASA impaired aggregation responses to ADP, epinephrine, and collagen, while ristocetin remained unaffected. Thrombotic patients showed further dysfunction. LTA parameters offered quantitative insights into platelets function, supporting the broader implementation and standardization in clinical laboratories of this technique.

Key words: platelets, light transmission aggregometry, acetylsalicylic acid, thrombosis, aggregation curve, ADP, collagen, epinephrine, ristocetin.

1. INTRODUCTION

Platelets (PLTs) are the smallest circulating blood cells, consisting of anucleate, discoid fragments derived from megakaryocytes, with a typical diameter of 2–5 μm [1, 2]. They play a critical role in hemostasis equilibrium by adhering to damaged vessel walls, aggregating, and facilitating clot formation. In PLTs function analysis, various aggregation techniques are used to evaluate platelet responsiveness to specific agonists. These include Light Transmission Aggregometry (LTA), Thromboelastography (TEG), Impedance Aggregometry, the VerifyNow assay, Platelet Function Analyzer (PFA-100), and flow cytometry [3-5]. Such tests are essential for diagnosing bleeding disorders and monitoring the efficacy of antiplatelet therapies.

LTA is a widely utilized technique for assessing PLTs aggregation function by monitoring changes in light transmission through platelet-rich plasma (PRP) upon

stimulation with specific agonists [4, 5]. To acquire an aggregation curve, hematology hospital laboratories are using standardized equipment which regularly provides only qualitative information about the PLTs aggregability in response to various agonists: aggregation present/absent/reduced or reversible.

Despite their apparent simple morphology, PLTs have a complex physiology. PLTs qualitative and/or quantitative defects can increase the risk of bleeding, while high residual platelet reactivity in patients receiving antiplatelet therapy contributes to thromboembolic complications [5]. Platelets have a short lifespan, averaging 8–10 days in circulation, necessitating continuous production to maintain a normal circulating blood range ($150\text{--}400 \times 10^9$ PLTs/l) [2]. This homeostatic production is remarkably consistent across individuals, regardless of age, sex or ethnicity [6].

When a bleeding injury occurs, the damaged tissue factors trigger the coagulation cascade and the activation of the platelets. Together, these processes ensure the rapid formation of a stable hemostatic plug composed of aggregated platelets and cross-linked fibrin [1, 5, 7]. Importantly, the alterations in platelet function – whether due to impaired activation, reduced aggregation, or structural changes – can significantly impact hemostatic competence and contribute to thrombotic or bleeding disorders [8].

Although LTA is the gold standard for diagnosing platelet function disorders, it has limitations due to sensitivity to preanalytical (e.g., anticoagulant type, hemolysis, low platelet count) and procedural factors (e.g., PRP preparation, agonist concentration) [9]. Accurate interpretation requires skilled personnel, and the method is subject to ongoing standardization [5, 9].

Up to date, there are only a few systematic quantitative studies about modifications of the aggregation curve parameters, in various pathological states or because of different medications [9, 10]. The aggregation curve yields several key parameters that reflect various aspects of platelet reactivity and aggregation dynamics. To interpret and compare the data, the recorded signal must be normalized as presented here:

$$\text{Aggregation (\%)} = \frac{T - T_0}{100 - T_0} \times 100\% \quad (1)$$

where T is the current light transmission and T_0 is the initial light transmission (before adding PLTs agonists). By doing this, all aggregation curves will start at 0 (baseline), no matter the exact platelets' concentration.

In Fig. 1, some important parameters of an LTA curve are shown. After the addition of an agonist, a negative deflection is observed, attributed to the PLTs shape changes (a marker of PLTs activation). When PLTs start to aggregate, light transmission increases. The time elapsed between the addition of the agonist and the start of the aggregation process is called lag phase time; it offers insights into early

activation kinetics.

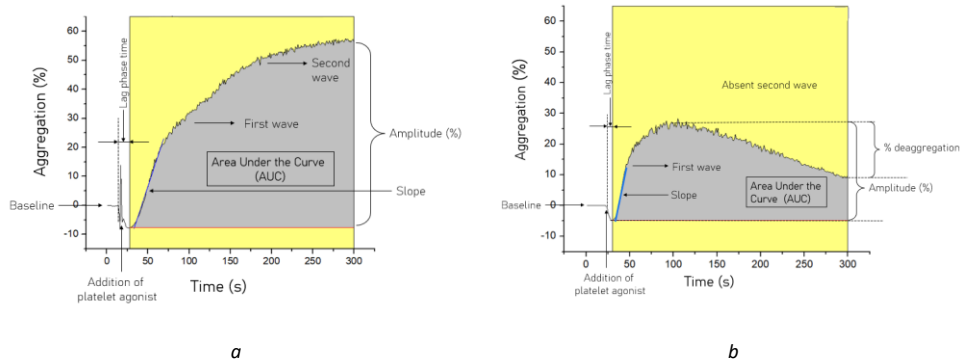


Fig. 1 – Example of aggregation curves and parameters which can be computed upon them: normal aggregation (a) and aggregation with deaggregation (b).

The slope, or aggregation rate (measured as the initial slope after the lag phase time, when the curve is quasilinear) reflects how quickly platelets respond to the agonist, indicating the aggregation speed. The amplitude represents the maximum extent of aggregation and correlates with the overall aggregation capacity. The area under the curve (AUC) integrates amplitude and duration, providing a cumulative measure of platelet responsiveness over time. In some cases (Fig. 1 b), after the aggregation reaches a maximum value, a deaggregation process may occur. The degree of deaggregation reflects the stability of platelet aggregates after maximum aggregation is reached, indicating whether platelet-platelet interactions are sustained or reversible.

An aggregation curve may have biphasic behavior. The first wave (primary phase) results from direct platelet activation and reversible aggregation due to weak agonist-receptor interaction [4]. This is followed by a second wave (secondary phase) driven by the autocrine/paracrine action of secondary mediators – most notably thromboxane A₂ (TXA₂) and adenosine diphosphate (ADP) – released from platelet dense granules. The second wave signifies irreversible aggregation and robust platelet activation [4]. In healthy individuals, both phases are typically present when stimulated with appropriate concentrations of weak agonists such as ADP or epinephrine. However, in the presence of strong agonists, such as collagen or ristocetin, a typical single, irreversible aggregation wave is observed [10]. In the latter cases, biphasic behavior is not seen due to the rapid initiation of the second wave of aggregation.

The purpose of the study is to highlight how parameters calculated on the aggregation curve (such as lag phase time, slope, amplitude, area under the curve (AUC), and degree of deaggregation) are influenced by certain conditions: intake of antiaggregant medication (acetylsalicylic acid, ASA) and thrombotic status.

ASA, widely known as aspirin, is a unique nonsteroidal anti-inflammatory drug with potent antithrombotic properties. It exerts its effect by irreversibly inhibiting the cyclooxygenase enzymes COX-1 and COX-2 through acetylation of key serine residues (Ser529 in COX-1 and Ser516 in COX-2) [11]. This inhibition blocks the conversion of arachidonic acid into prostaglandin H₂ (PGH₂), a precursor of thromboxane A₂ (TXA₂) – a potent chemoattractant for PLTs aggregation. Since platelets primarily express COX-1, ASA markedly reduces TXA₂ synthesis, impairing PLTs aggregation and thereby lowering the risk of thrombotic events [11]. Following ASA administration, PLTs aggregation is permanently reduced, diminishing platelets responsiveness to pro-aggregatory stimuli. Due to the absence of a nucleus, platelets cannot synthesize new COX enzymes, rendering the inhibitory effect of ASA irreversible for the lifespan of the platelet [12]. This irreversible action forms the basis for ASA's long-standing role as a cornerstone in antiplatelet therapy for the prevention of thrombotic complications in cardiovascular patients [13]. Thrombosis refers to the pathological formation of blood clots within blood vessels and represents a major clinical concern in patients with cardiovascular disease [14]. This cardiovascular disease is more prone to thrombotic events necessitating thus targeted antiplatelet therapy [15].

The result of the study can be used as a basis for further recommendations and accreditation of laboratory practice. Platelet aggregation was measured on a control group of healthy volunteers before and after receiving ASA and on patients with a history of thrombotic events who were receiving daily ASA therapy.

2. METHODS

2.1. PATIENTS

Three groups of patients were recruited for the study: i) 39 healthy volunteers before ASA intake (C-ASA); ii) among which 19 healthy volunteers were monitored also after completing a 14 days-ASA treatment (C+ASA); iii) 5 cardiovascular patients with thrombotic history (T). The choice of a 14-day period of ASA ingestion was made considering the lifespan of circulating platelets. The thrombotic events included arteriopathy, acute myocardial infarction, and cerebrovascular stroke. All

thrombotic patients were receiving ASA as standard of care when included in the study. Thrombotic patients were enrolled based on a confirmed diagnosis established using the criteria of International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10) and were in a clinically stable condition at the time of inclusion [16]. Other relevant inclusion and exclusion criteria were normal values of routine laboratory assessments (performed within four weeks prior to enrollment) [17].

Following approval by the Ethics Committee of Colentina Clinical Hospital, this study was performed in accordance with the Declaration of Helsinki, and written informed consent was obtained from all participants.

2.2. PREPARATION OF PLTS SUSPENSION

Whole blood was collected by venipuncture into vacutainers containing 0.0129 M sodium citrate as anticoagulant. Platelet Rich Plasma (PRP) was obtained by gentle centrifugation (150 x g, 10 min, room temperature). Platelet-Poor Plasma (PPP) was separated by further centrifugation at 2000 x g for 15 minutes (room temperature) of the remaining sample after PRP collection. This PPP was used as blank.

2.3. MEASUREMENT OF PLTS AGGREGATION

PLTs aggregation was assessed using a UV-Vis spectrophotometer (Jasco V-600, UK). The blank light transmission was measured at 633 nm using two cuvettes with 500 μ l PPP each. Then, one of the cuvettes was replaced with a cuvette containing 500 μ l of PRP, the PLTs suspension being kept at 37°C for 2 minutes. The light transmission record was set for 300 seconds. At the 20-second mark, aggregation was induced by injecting into the PRP cuvette a volume of aggregation agent. Four types of agonists have been used: 5 μ M ADP (Chrono-Log, 384), 10 μ M epinephrine (Chrono-Log, 393), 4 μ g/ml collagen (Chrono-Log, 385), and 1.25 mg/ml ristocetin (Chrono-Log, 396). The normalized aggregation curve was computed to extract the following parameters: lag phase time, slope, amplitude, area under the curve (AUC), and degree of deaggregation, parameters detailed in Fig. 1. For an easier interpretation of the results, the discussion about % deaggregation will further consider the absolute value of this parameter.

2.4. STATISTICAL ANALYSIS

Data analysis was conducted using descriptive statistics, the median test, and the Kruskal–Wallis test for variables with non-normal distributions, as determined by the Shapiro–Wilk normality test. All statistical analyses were performed using SPSS version 27 (IBM Corp.), significance levels are denoted as follows: $p < 0.05$ (*), $p < 0.01$ (**).

3. RESULTS

Platelet aggregation responses to all types of agonists used (ADP, epinephrine, collagen, and ristocetin) were evaluated for all groups described before (C-ASA, C+ASA, and T). The aggregation parameters analyzed were lag phase time, slope, amplitude, area under the curve (AUC), and degree of deaggregation, parameters detailed in Fig 1.

3.1. PLTs AGGREGATION INDUCED BY ADP

Compared to the C-ASA group, after 14 days of ASA intake (C+ASA), platelet aggregation of healthy volunteers was reduced, as reflected by a tendency of decreased amplitude (Fig. 2 d) and a significant decreased AUC (Fig. 2 e). Moreover, a significant higher % deaggregation was found (Fig. 2 f), indicating the inhibition of the second phase of aggregation by ASA.

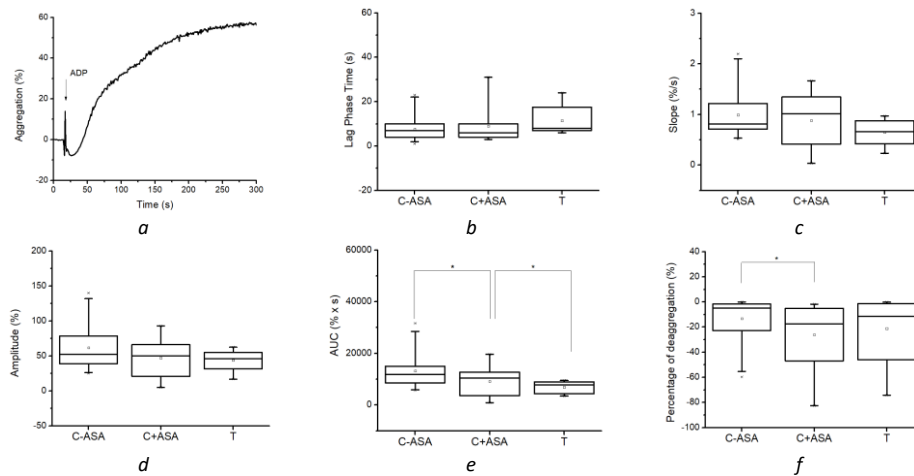


Fig. 2 – Example of normal ADP induced platelet aggregation curve (a); b-f: aggregation curve parameters computed for healthy controls (without [C-ASA] and with ASA intake [C+ASA]) and for thrombotic patients [T].

In thrombotic patients (T), who received ASA as a daily treatment, the aggregation profile showed a significantly reduced AUC (Fig. 2 e) compared to the C+ASA group ($p < 0.05$). This suggests that the thrombotic status supplementary induces a reduced responsiveness of platelets to ADP.

3.2. PLTs AGGREGATION INDUCED BY EPINEPHRINE

In this case, a comparative analysis between the C-ASA and C+ASA groups revealed no significant differences in all parameters of the PLTs aggregation curve (Fig. 3). One may still observe a larger range of values for % deaggregation suggesting a more frequent absence of the second wave of aggregation at healthy volunteers with ASA intake.

When comparing thrombotic patients (T) with the C+ASA group, there were statistical differences for certain parameters. The lag phase time was significantly prolonged in the T group compared to C+ASA (Fig. 3 b), suggesting either enhanced platelet inhibition or intrinsic platelet dysfunction. Even if there is not statistically significant, a tendency of diminished slope, amplitude, and AUC (Fig. 3 c, d, e, respectively) was observed in thrombotic patients, indicating a near-complete lack of aggregation and thus a nearly absent % deaggregation (Fig. 3 f). Interestingly,

while C+ASA individuals still retained an aggregation response, thrombotic patients exhibited a blunted and non-reversible aggregation profile.

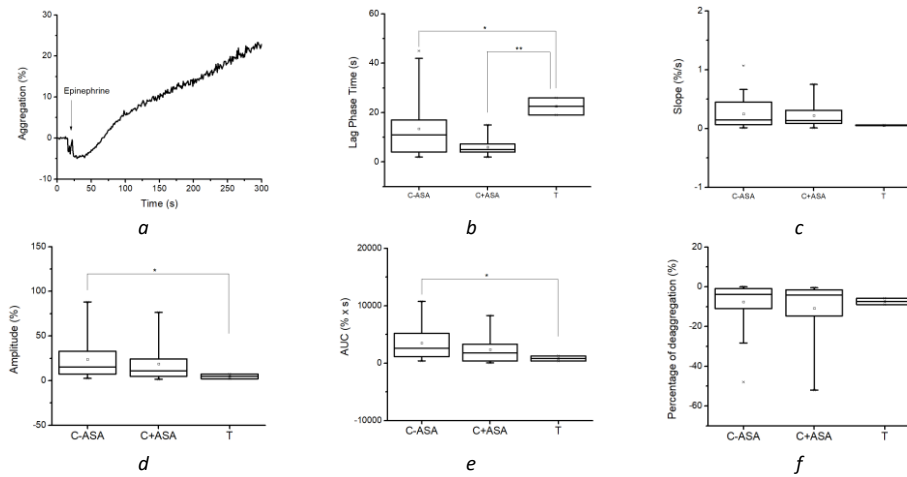


Fig. 3 – Example of normal epinephrine induced platelet aggregation curve (a); b-f: aggregation curve parameters computed for healthy controls (without [C-ASA] and with ASA intake [C+ASA]) and for thrombotic patients [T].

3.3. PLTs AGGREGATION INDUCED BY COLLAGEN

In the C-ASA group, collagen stimulation induced, as expected, a strong and stable aggregation response, reflected in high amplitude and AUC values (Fig. 4 d and e) compared to aggregation induced by weaker agonists: ADP (Fig. 2 d and e) and epinephrine (Fig. 3 d and e). Following ASA intake (C+ASA), platelet aggregation parameters presented no significant differences compared to those of C-ASA group, even though one may observe a tendency of lower median values of amplitude and AUC parameters, together with a larger range of values of % deaggregation.

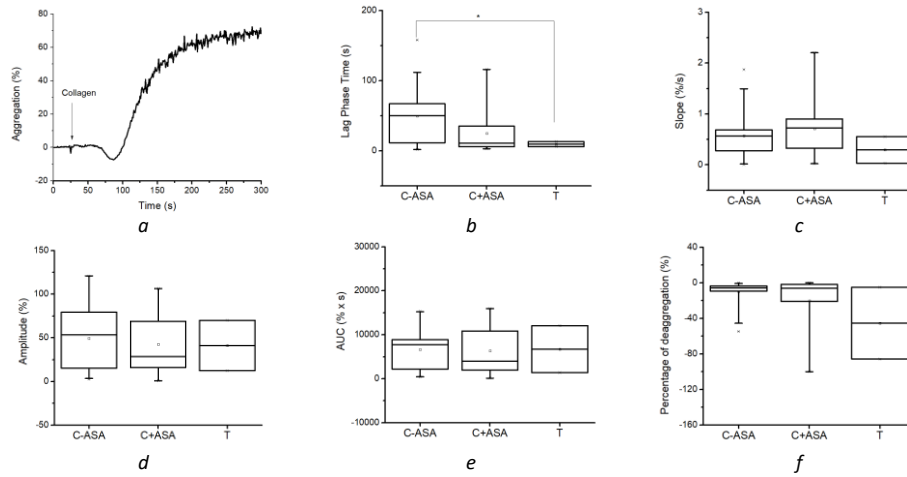


Fig. 4 – Example of normal collagen induced platelet aggregation curve (a); b-f: aggregation curve parameters computed for healthy controls (without [C-ASA] and with ASA intake [C+ASA]) and for thrombotic patients [T].

The thrombotic patients (T), who take ASA as daily treatment, had no significant modification of aggregation parameters when compared to C+ASA. A tendency of larger range of values of % deaggregation observed in the case of T patients may be caused by the presence of ASA treatment, suggesting rapid yet insufficient platelet activation, possibly reflecting prior antithrombotic therapy or underlying alterations in platelet function.

3.4. PLTs AGGREGATION INDUCED BY RISTOCETIN

In the C-ASA group, ristocetin-induced aggregation yielded consistent amplitude and AUC values (Fig. 5 d and e) with minimal deaggregation (Fig. 5 f) compared to the other strong agonist, collagen (Fig. 4 d, e and f), indicating normal vWF–GPIIb function. After 14 days of ASA intake (C+ASA), aggregation responses remained largely unchanged across all parameters. This is consistent with the mechanism of ASA, which primarily targets cyclooxygenase-1 (COX-1) and does not interfere directly with vWF–GPIIb-mediated adhesion. In thrombotic patients (T), aggregation responses to ristocetin were like those observed in C+ASA group.

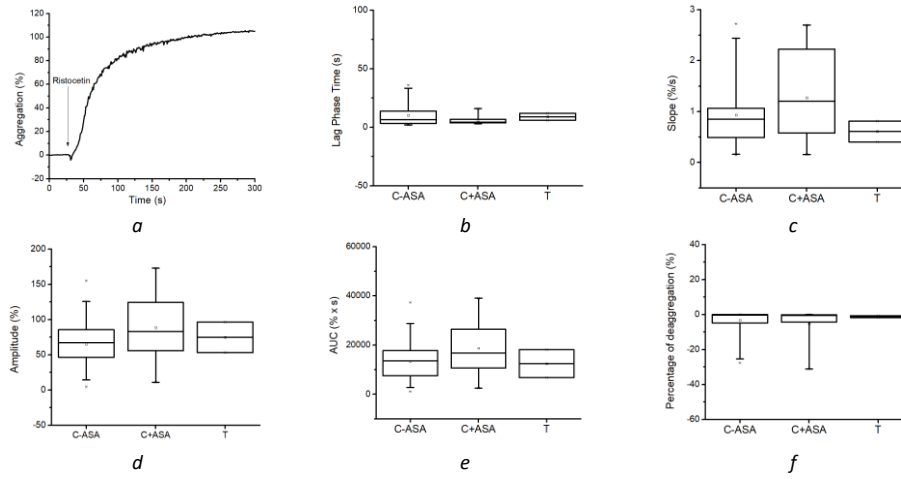


Fig. 5 – Example of normal ristocetin induced platelet aggregation curve (a); b-f: aggregation curve parameters computed for healthy controls (without [C-ASA] and with ASA intake [C+ASA]) and for thrombotic patients [T].

4. DISCUSSION

Different agonists are generally used in LTA tests to assess the molecular pathways of platelets activation. ADP induces platelet aggregation via $P2Y_1$ and $P2Y_{12}$ receptors, leading to PLTs shape change and granules content release [18]. Epinephrine acts through α_2 -adrenergic receptors, determining the release of ADP from intracellular dense granules, process which is then followed by an ADP-induced aggregation response [19]. Collagen activates platelets through GPVI and integrin $\alpha_2\beta_1$, simulating vascular injury and promoting strong, sustained aggregation [20]. Ristocetin, unlike the other agonists, does not induce aggregation via activation of receptors but facilitates PLTs agglutination through von Willebrand factor (vWF) which bind to GPIb receptor, thus serving as a diagnostic tool for vWF-related disorders [19].

The present findings revealed a differential inhibitory effect of acetylsalicylic acid on PLTs aggregation in response to various agonists. The pharmacological action of ASA, which irreversibly inhibits cyclooxygenase-1 (COX-1), is leading to the suppression of thromboxane A_2 (TXA₂) synthesis causing the inhibition of this secondary mediator in PLTs activation pathways, resulting mainly in absence of the

second wave. In the present study, this effect was demonstrated by the increased absolute values of % deaggregation parameter in the cases of PLTs aggregation curves triggered by ADP, epinephrine, and collagen. A more pronounced inhibitory effect on aggregation was induced in the case of ADP, where one may also observe a decrease of amplitude and AUC values.

In case of ristocetin tests, no significant differences were observed among groups for any computed aggregation parameter, indicating a preserved functionality of the von Willebrand factor (vWF)–GPIIb axis. Since ristocetin-induced aggregation does not depend on the COX-1/TXA₂ pathway, the fact that ristocetin-induced aggregation was unaffected by ASA, was anticipated. Ristocetin-based tests remain clinically important and reliable in diagnosing PLTs function disorders such as von Willebrand disease, Bernard–Soulier syndrome, and Glanzmann thrombasthenia, where aggregation is typically absent or severely impaired. Although some reports have suggested potential ASA-induced modulation of ristocetin responses under certain conditions (aggregation measurements using whole blood) [21], no such effect was observed under the current experimental settings using PRP.

In platelet-rich plasma type of sample, at concentrations below 1 μM, ADP typically induces a reversible primary wave of PLTs shape change and aggregation, which is prone to deaggregation. A complete aggregation response to ADP is achieved at higher ADP concentrations (5–10 μM). A biphasic aggregation response occurs (Fig. 1 a), presenting a second phase requiring de novo TXA₂ synthesis. Willing to capture an effect of ASA on the aggregation parameters, a 5 μM concentration of ADP was chosen for present study. In PRP, epinephrine does not induce PLTs shape change but can elicit a biphasic aggregation curve at concentrations above 5 μM, with a second phase similarly dependent on TXA₂ synthesis. A 10 μM concentration of epinephrine was chosen for present study and thus an inhibitory effect of ASA was observed, as mentioned above.

The concentration of the collagen used in present study was 4 μg/ml, concentration within the range of values for a normal aggregation test (1–5 μg/ml). At low collagen concentrations (1–2 μg/mL) ASA intake of 250 mg daily is shown to reduce the PLTs aggregation, while at higher concentrations (5 μg/mL) this effect is generally overcome through alternative activation mechanisms [22]. Remarkably is that in present study, the inhibitory effect of ASA was revealed even for low daily dosages of only 75 mg.

This study shows that thrombotic patients (T) on daily ASA had generally altered platelet responses compared to ASA-treated controls (C+ASA). ADP-induced aggregation (in terms of AUC values) was significantly reduced in T

patients, indicating lower overall platelet responsiveness. With epinephrine, T patients showed a significant longer lag phase and trends toward reduced slope, amplitude, and AUC, suggesting stronger PLTs dysfunction. Collagen responses were similar between groups, but T patients had more variable absolute values of % deaggregation. Ristocetin-induced aggregation remained stable across all groups, consistent with ASA's mechanism, which does not affect vWF–GPIb interactions. Overall, thrombosis appears to impair specific platelet pathways beyond ASA's effects, especially those activated by ADP and epinephrine. These results emphasize the complex interactions between pharmacological inhibition, platelet physiopathology, and therapeutic interventions.

The LTA (light transmission aggregometry) test provides valuable information that supports its inclusion as a standard method for platelet function assessment. Starting with 2008, international scientific societies dedicated to thrombosis and hemostasis recommended standardization of LTA test protocols mainly in terms of agonists' concentrations. Despite inherent limitations of the technique (such as logistical hurdles, technical and interpretive demands of results analysis and less clinical correlation) [20], LTA offers detailed insights into both the first and second phases of platelet aggregation as quantitative data. Our study contributes to the growing body of arguments [23] that promote/propose the LTA test as regular practice in hematology laboratories when analytical PLTs function analysis (e.g., % deaggregation) is necessary. The multifactorial nature of platelet activation and aggregation requires the necessity of accreditation/standardization of the LTA evaluation in the perspective of personalized diagnostic approaches in clinical practice.

5. CONCLUSIONS

The present study demonstrates that Light Transmission Aggregometry (LTA) provides a detailed, quantitative assessment of platelet function, revealing subtle alterations in aggregation dynamics in response to different agonists under the influence of acetylsalicylic acid (ASA) and thrombotic conditions. ASA intake, even at low doses (75 mg/day), significantly impairs platelet aggregation by inhibiting the COX-1/TXA₂ pathway, particularly affecting the PLTs aggregation response to ADP, epinephrine and collagen, while leaving ristocetin-induced aggregation unchanged. Thrombotic patients on ASA therapy exhibited further impaired platelet responsiveness compared to controls with ASA intake, suggesting an additional

platelet dysfunction inherent to the thrombotic state. These findings highlight the importance of assessing key parameters computed from PLTs aggregation curve (such as lag phase time, slope, amplitude, AUC, and % deaggregation) for a nuanced understanding of platelet behavior in both healthy and pathological contexts. Given its capacity to detect both reversible and irreversible aggregation phases and to differentiate the effects of pharmacologic and pathologic factors, LTA remains a valuable tool in platelet function testing. This study supports the continued standardization and broader implementation of LTA as a gold standard in hematology laboratories, particularly for personalized diagnostics in patients receiving antiplatelet therapy or presenting with thrombotic risk.

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Ethics statement. The study was carried out in accordance with national and institutional ethical standards. Ethical approval was granted by the Ethics Committee of Colentina Clinical Hospital (Approval No. 6/10.01.2017), including approval of the study protocol, participant information materials, and informed consent forms.

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