Classic Light Transmission Platelet Aggregometry: Do We Still Need it?

Jennifer Gebetsberger[1] Florian Prüller[2]

[1] Department of Pediatrics I, Medical University of Innsbruck, Innsbruck, Austria
[2] Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria

Address for correspondence Florian Prüller, Dr. med. univ. Florian Prüller, MD, Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Auenbruggerplatz 15, 8036 Graz, Austria (e-mail: florian.prueller@medunigraz.at).

Hamostaseologie

Abstract

For more than 50 years, light transmission aggregometry has been accepted as the gold standard test for diagnosing inherited platelet disorders in platelet-rich plasma, although there are other functional approaches performed in whole blood. In this article, several advantages and disadvantages of this technique over other laboratory approaches are discussed in the view of recent guidelines, and the necessity of functional assays, such as light transmission aggregometry in the era of molecular genetic testing, is highlighted.

Keywords

- blood platelet disorders
- platelet function tests
- platelet aggregation

Zusammenfassung

Seit mehr als fünfzig Jahren gilt die Aggregometrie mittels Lichttransmission als Goldstandard-Test zur Diagnose von erblichen Blutplättchenstörungen in Plättchenreichem-Plasma, obwohl es noch weitere funktionelle Testansätze in Vollblut gibt. In diesem Artikel werden mehrere Vor- und Nachteile dieser Technik im Vergleich zu anderen laborbasierten Ansätzen im Hinblick auf aktuelle Leitlinien diskutiert, und die Notwendigkeit von funktionellen Assays, einschließlich der Aggregometrie mittels Lichttransmission, im Zeitalter der molekulargenetischen Tests wird hervorgehoben.

Schlüsselwörter

- Blutplättchenstörungen
- Tests zur Plättchenfunktion
- Thrombozytenaggregation

Introduction

Platelets play a pivotal role in normal hemostasis and in pathological thrombosis. They are recruited to the site of vascular wall injury or ruptured atherosclerotic plaque through the adhesive interactions between platelet glycoproteins and integrins with von Willebrand factor and subendothelial collagen.[1] In response to collagen and soluble agonists, such as adenosine diphosphate (ADP) and thrombin, platelets undergo activation, leading to the formation of a stable thrombus via two simultaneous processes. Activation of integrin αIIbβ3 (also known as GPIIb/IIIa) results in the formation of platelet–platelet homotypic aggregates via fibrinogen molecular bridges, forming a physical barrier at the site of injury.[2] In response to strong stimulation, a subset of activated platelets exposes negatively charged procoagulant phosphatidylserine (PS) on their surface and supports generation of thrombin. The thrombin burst localizes the formation of fibrin to the platelet aggregate, thereby stabilizing the clot. Therefore, platelet aggregation and procoagulant activity interplay to form a platelet–fibrin thrombus.[3,4]

In clinical settings, evaluation of platelet function is vital for detecting both inherited and acquired qualitative platelet deficiencies. It is also used to diagnose systemic bleeding disorders (►**Fig. 1**). Platelet function tests also play a role in monitoring the efficacy of antiplatelet/antithrombotic medications, which are widely used in treating cardiovascular diseases. Anesthesiologists and surgeons often order such tests as a means to screen for platelet-related bleeding disorders prior to invasive procedures and interventions.[5–8]

received
April 28, 2023
accepted after revision
October 6, 2023

Georg Thieme Verlag KG,
Rüdigerstraße 14,
70469 Stuttgart, Germany

DOI https://doi.org/10.1055/a-2117-4614.
ISSN 0720-9355.

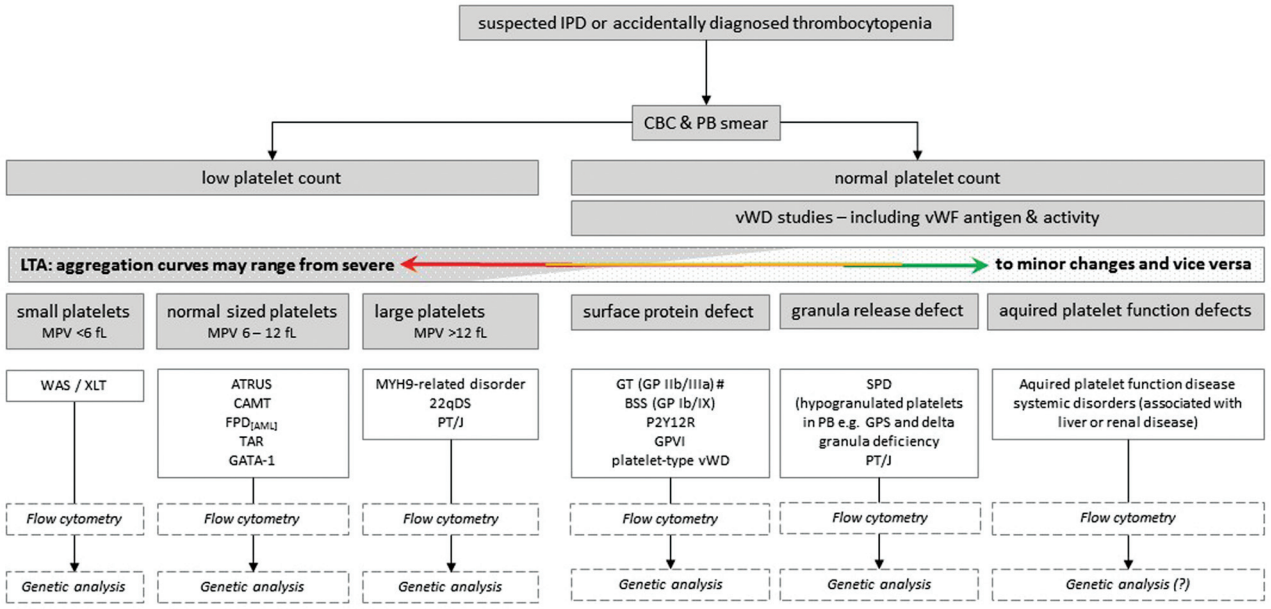

Fig. 1 Diagnostic pathway to (potentially) identify inherited platelet disorders. The diagnostic pathway outlines the procedure for using various technical approaches to diagnose and confirm different platelet defects. Classic LTA patterns may range from severe to minor irregular aggregation curves regardless of the final underlying diagnosis. This variability is depicted in a box entitled "Aggregation curves may range from severe to minor changes and vice versa" depending on the different agonists. An arrow transitioning from red to orange to green emphasizes that there are patterns depending on the agonist and these patterns may be independent of maximum amplitude and the area under the curve (AUC). Acquired platelet function deficits may occur due to various anti-platelet antibodies (e.g., sepsis associated) or due to various synthetic and herbal drugs (i.e., antiplatelet therapy or dietary supplements). Flow cytometry and genetic analysis (italicized, dashed lines) may be performed depending on the availability of diagnostic resources. 22qDS, 22q deletion syndrome; ATRUS, amegakaryocytic thrombocytopenia with radioulnar synostosis; BSS, Bernard-Soulier syndrome; CAMT, congenital amegakaryocytic thrombocytopenia; CBC, complete blood count; $FPD_{[AML]}$, familial platelet disorder with predisposition to acute myeloid leukemia; GP, glycoprotein; GPS, gray platelet syndrome; IPDs, inherited platelet disorders; LTA, light transmission aggregometry; MPV, mean platelet volume; P2Y12R, P2Y12 receptor deficiency; PB, peripheral blood; PT/J, Paris-Trousseau/Jacobsen syndrome; SPD, storage pool disease; TAR, thrombocytopenia with absent radii; TXA2R, thromboxane A2 receptor deficiency; vWD, von Willebrand disease; vWF, von Willebrand factor; WAS, Wiskott-Aldrich syndrome; XLT, X-linked thrombocytopenia. Modified from various studies.[15,17,28,150]

Among all the methods for platelet function testing,[9–12] classic light transmission aggregometry (LTA) remains a historical reference method and continuous to be used extensively. In this article, we describe the principles and adaptations of this method, and discuss its advantages and disadvantages compared with alternative platelet function tests, particularly in relation to the inherited disorders in pediatric populations. Furthermore, we strive to answer the question: Is this method still necessary or has it become outdated?

Method

A literature search strategy was developed using the PubMed platform of the National Center for Biotechnology Information (NCBI). The search included peer-reviewed articles published in English and German from January 2013 to January 2023. We utilized the MeSH terms "blood platelet disorders" *and* "platelet function tests" *and* the search terms "platelet rich plasma" (38 results) *or* "whole blood" (186 results), respectively. In total, 224 references were identified and screened for their relevance and quality (►**Supplementary Fig. S1** [online only]). From these, a total of 66 articles were considered and supplemented by guidelines from various medical associations (i.e., *British Society for Haematology*,[13] *British Committee for Standards in Haematology*,[14] *Subcommittee on Platelet Physiology of the International Society on Thrombosis and Hemostasis*,[15] *Permanent Pediatric Committee of the Society of Thrombosis and Haemostasis Research*,[16] and AWMF guideline 086–003: diagnosis of platelet function disorders—thrombocytopathies[17]).

Additional insights are drawn from the corresponding author's practical experience as a consultant and laboratory medicine specialist focusing on coagulation testing. The author oversees the diagnosis of inherited and acquired coagulation disorders in the outpatient service and manages coagulation testing performed in the central laboratory of the University Hospital Graz, Medical University Graz (Austria). Further scientific information was obtained through personal communications within the working group THROMKIDplus during the pediatric GTH meeting (pedGTH) held in September 2022 in Igls, Innsbruck (Austria).

How to Diagnose an Inherited Platelet Disorder?

A positive (pediatric) ISTH Bleeding Assessment Tool (BAT) Score[18] alongside plasmatic coagulation tests without pathological findings guides to the suspicion toward platelet-associated diseases, mainly inherited platelet function disorders (IPFDs).[19] To date, ~60 types of inherited platelet disorders (IPDs) have been identified, caused by molecular defects in ~75 different genes.[20,21] The severity of these disorders varies widely, ranging from mild symptom

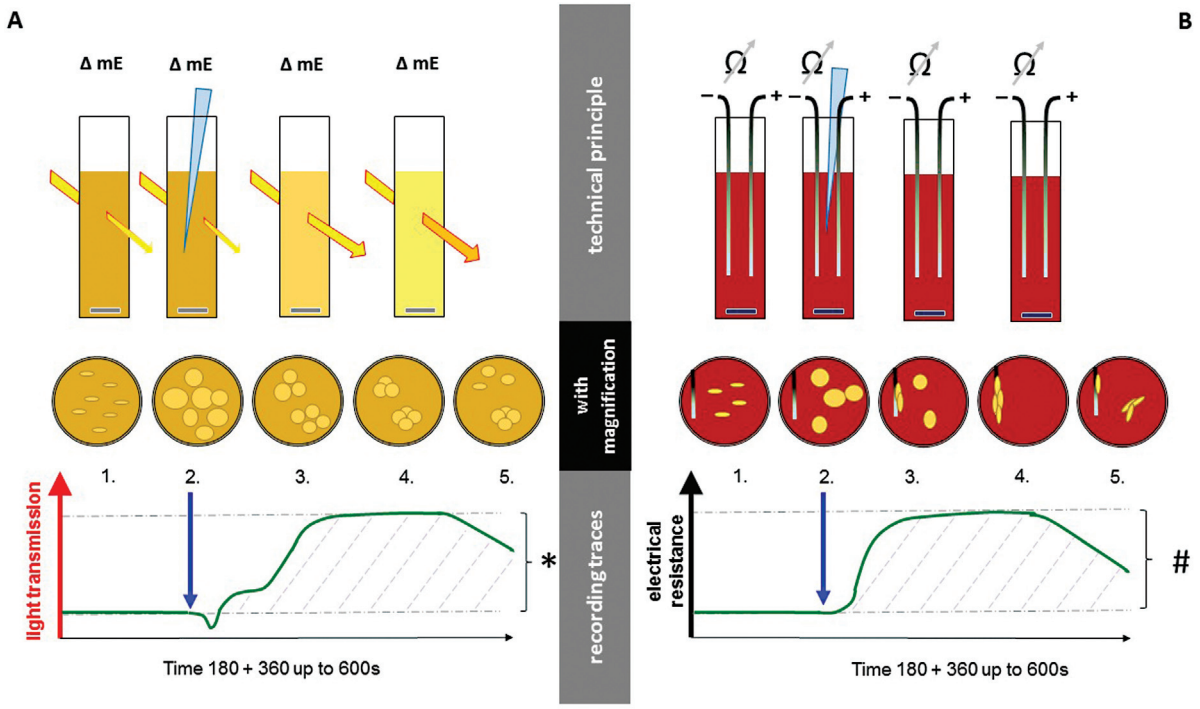

Fig. 2 Principle of light transmission aggregometry and whole blood impedance aggregometry. (**A**) Top row [technical principle] shows the cuvette with platelet-rich plasma (PRP) and a stirring bar, light source, and light detection system (not drawn). The difference in milli extinction (Δ mE) is measured upon addition of an agonist (indicated by blue pipette tip). (**B**) Top row [technical principle] with whole blood (WB), a stirring bar and electrodes to record the change in resistance (Ω↗) upon addition of an agonist (indicated by blue pipette tip). Middle row [with magnification] shows how the platelets react in PRP (A) and WB (B): resting platelets (1) changing conformation and volume (i.e., "shape change") after adding agonist (2), which is not recorded in WB; formation of in-stable aggregates (1st wave) (3); formation of stable aggregates (2nd wave) (4), and sometimes disaggregation (5) in PRP and only in selected devices in WB. Bottom row [recorded tracings] shows the recorded tracings over time in PRP (A) and WB (B) calculating maximum amplitude (%, curly brackets) based on the Δ of the transmitted light (*) or the Δ of electrical resistance (#) and area under the curve (AUC, dashed light purple lines), respectively. Adding luciferase reagent to PRP or WB allows recording the release of ATP in an additional channel in selected devices.

presentations to severe bleeding disorders such as Glanzmann's thrombasthenia and Bernard-Soulier syndrome. Defects can occur in platelet receptors, glycoproteins, granular release or content, transcription factors, and signaling pathways.[22] Due to the complexity of these diseases, the diagnostic workup employs a broad spectrum of methods and procedures, the accessibility of which varies across specialized clinical laboratories.[23,24] As advised by the International Society on Thrombosis and Haemostasis Subcommittee on Platelet Physiology (ISTH-SSC),[18,25] the British Society for Haematology (BSH),[13] the "Gesellschaft für Thrombose- und Hämostaseforschung" (GTH e.V.),[17] and as performed in various countries (e.g., Northern Europe,[26] Italy,[27] Asia,[28] Australia[29]), the diagnosis of platelet disorders should involve a step-wise approach (►**Fig. 1**).

Classic Light Transmission Aggregometry

A key method in diagnosing platelet functional disorders is LTA. This technique was independently developed in the 1960s by Born and O'Brien,[30–32] and is still considered the gold standard diagnostic approach for monitoring both physiologic and pathophysiologic platelet functions. It has proven performance characteristics and can detect abnormalities associated with increased bleeding in a significant proportion of individuals referred for platelet function investigations. The principle of this method is based on the increase in light transmission with platelet aggregation. Photometric measurements are therefore conducted on 200 to 500 µL suspensions of platelets in plasma (platelet-rich plasma [PRP]) or in buffer (washed platelets or gel-filtered platelets). These measurements are performed secondary to platelet aggregation, which is induced by a variety of relevant activators (►**Fig. 2**). Typical agonists in various concentrations used to stimulate platelets include ADP, collagen, epinephrine, ristocetin, thrombin peptides (e.g., thrombin receptor activating peptide 6 [TRAP-6]), and arachidonic acid, among others. These agonists activate platelets by binding to specific receptors at the platelet surface, which leads to a series of downstream events that ultimately increases the intracytoplasmic concentration of calcium ions in the platelets. This mobilization of calcium ions prompts the release of platelet granules, facilitating the local release of small molecules from the platelets. These molecules attract additional platelets, leading to interplatelet connections and aggregation (►**Fig. 2**). The flexibility of LTA stems from the ability to use different agonists in various concentrations, allowing the identification of platelet shape change, platelet de-aggregation, or the occurrence of secondary wave aggregation.[33] Commercially available testing devices and solutions are listed in ►**Table 1**. Several studies confirm good or excellent inter-method correlation among the different devices.[34]

Table 1 Commercially available devices for the detection of acquired or inherited platelet defects (PD), a non-exhaustive list

	Platelet Rich Plasma based method				Whole Blood based method				
	Company	Device	Reagents	Application	Company	Device	Reagents	Application	
manual	Chronolog	490 and 700[a]	user's choice	PD, monitoring	Chronolog	WBA and 700[a]	user's choice	PD, monitoring	manual
	Helena	AggRAM	user's choice	PD, monitoring	Roche	Multiplate	fixed set of reagents	monitoring	
	Biodata	PAP 8	user's choice	PD, monitoring	Haemonetics	TEG 5000	fixed set of reagents	monitoring	
	Labitec	APACT 4004	user's choice	PD, monitoring	Werfen	ROTEM platelets	fixed set of reagents	monitoring	
	Stago	TA-V4 & 8	user's choice	PD, monitoring	Siemens Healthineers	PFA 100 / 200	cartridge	PD, monitoring	semi-automated
automated	Siemens Healthineers	COAG 360[b]	user's choice	PD, monitoring	Werfen	VerifyNow	cartridge	monitoring	
	Sysmex	CS-2500 CS-5100 CN-3000 CN-6000	user's choice	PD, monitoring	Matis Medical	Cone and Plate(let) Analyzer (CPA) Impact R [RUO]	cartridge	monitoring	
	Behnk Elektronik	Thrombomate XRA	user's choice (?)	PD, monitoring					

[a]This device allows for adenosine triphosphate (ATP) secretion testing.
[b]This device is to be removed from the market in Q3/2024 and CS and CN devices will be distrubuted in cooperation with Sysmex.

Manually performed LTA is time-consuming, leading to long turn-around time in daily clinical practice. In addition, it requires the fresh collection of a relatively large blood volume, presenting a particular challenge for young patients. Whole blood sample volume requirements, ranging from 10 to 20 mL, exceed what a neonatal patient can safely provide. Additionally, LTA is technically challenging, as it is influenced by several pre-analytical and analytical variables. As such, there is scientific controversy concerning the use of PRP versus whole blood,[35] the impact of anticoagulants (e.g., DOACs interfere with thrombin-dependent platelet function,[36] but these effects are often overlooked because exogenous thrombin[36] is added in excess in most LTA protocols, as well as the influence of unfractionated heparin[37]), centrifugation, transportation,[38] hands-on or turnaround time,[39] comparability between centers, adjusting platelet count,[40,41] standardization of used agonist, and quality control policies.[42–44] Moreover, the lowest reliable platelet count ranges from 30,000 to 100,000/µL,[45,46] which limits the analysis in patients with thrombocytopenia, a condition often found in IPD patients. Efforts to address and harmonize these issues have been made in published guidelines.[13,15,17,47]

Automated Light Transmission Aggregometry

New coagulation analyzers on the market enable the standardized performing of automated LTA, with reduced sample volume requirements (~140 µL PRP per test compared with 200–500 µL in classic LTA), reduced turnaround times, and without the need for dedicated, experienced personnel.[48–51] Despite these advantages, the costs of reagents and consumables for automated LTA may be higher than for classic LTA. Additionally, interpretation still depends on expert examination of aggregation tracings from a patient, in comparison to a healthy control.[52]

Another automated LTA variant is the high-throughput 96- to 384-well-based platelet function assay, which allows for a much broader overview of platelet function in significantly less time and with a reduced PRP volume requirement (50–100 µL per test for 96-well plates, 10 µL per test for 384-well plates). A large number of simultaneous aggregations can be run on the same plate, making it easy to generate concentration–response curves to numerous agonists. However, micro-plate readers do not mix the platelets in the same manner as classic aggregometers, and these readers cannot currently monitor changes in absorbance with mixing. Therefore, unlike classic LTA, 96-well and 384-well plate aggregometry cannot be used as a kinetic assay and, in our own experience, only worked well for functionally inconspicuous platelets. Additionally, as the physical forces acting on platelets are key determinants of their responses to agonists, the data derived from the two assays are not interchangeable.[53,54]

However, it is plausible that such automated assays could be administered in non-specialized centers, with the results then sent to a tertiary center for interpretation. The standardization of these automated, commercially available, and registered assays could help to alleviate some of the issues surrounding reproducibility among laboratories worldwide.

Impedance or Multiple Electrode Aggregometry

The impedance aggregometer was first described in 1980.[55] This device measures platelet aggregation by monitoring changes in electrical impedance (Ω↗) in PRP, and its use has been extended to include whole blood samples.[46,56] The measurement principle involves adding an agonist to stimulate platelets, which then aggregate and cover the electrodes, altering the electrical current conduction between them by creating an insulating platelet layer, and thus increasing electrical resistance (►**Fig. 2**). One main disadvantage of the first whole blood aggregometers, commercialized as the Chrono-log (Havertown, PA), was that the two electrodes used had to be carefully cleaned between analyses, which was largely considered as impractical for clinical use and introduced a potential source of error. On the other hand, disposable gold-plated electrodes have not established themselves for price reasons. Due to construction differences between the original whole blood aggregometers and later further reproduced devices, a decrease in resistance may be observed only in the former, sparking debates about their comparability.

Accordingly, the development of new, semi-automated systems has allowed wide uptake of these instruments in hematology laboratories, especially for P2Y12 inhibitor monitoring.[57] While there is some evidence to support the use of impedance aggregometry for diagnosing severe platelet function disorders,[58–60] this technique has been found to be less effective than classic LTA in detecting and differentiating mild platelet function disorders.[61–63] This is due to its inability to provide information about platelet shape changes and reversibility of aggregation. Additionally, as already described for the other techniques, several pre-analytical and analytical variables can affect the results provided by the instrument. These include the time interval between blood drawing and analysis, the type of anticoagulant used, and the platelet count.[64–66] Consequently, this technique is not currently recommended as a screening test for diagnosing platelet function disorders. However, for assessing the residual effect of antiplatelet therapy prior to surgery, the hypersensitivity of impedance aggregometry exceeds that of LTA.[67]

Lumi-Aggregometry

A modification of the LTA and multiple electrode aggregometry is the light transmission lumi-aggregometry (measuring PRP) and the whole blood impedance lumi-aggregometry, respectively, which measure platelet delta-granule adenosine triphosphate (ATP) secretion in parallel with platelet aggregation.[68] The assays are performed by adding a luciferin–luciferase reagent to a sample along with an agonist (e.g., collagen, thrombin), while stirring the sample at low shear to promote platelet activation and aggregation. The ATP released from platelets reacts with the luciferin–luciferase reagent, resulting in light emission that is usually quantified by a lumi-aggregometer, relative to an ATP standard. The advantages of the whole blood impedance lumi-aggregometer is that its assay milieu replicates in vivo platelet activation conditions, improved reproducibility, and near-patient testing.[69] This combined analysis thus enhances the detection of platelet disorders affecting dense granule release. However, this method is also affected by several variables, including the concentrations of luciferin/luciferase, agonists and ATP standard, sample volume, incubation time, duration of measurement, and adjustment of platelet count.[52]

Alternative Methods

Flow Cytometry

Another popular technique for platelet phenotyping is flow cytometry.[70,71] Unlike LTA, which measures the net result of multiple activation processes, flow cytometry allows for a more comprehensive exploration of platelet function. It can evaluate the presence, absence, or even the functionality of specific glycoproteins on both resting and activated platelets at a single cell level.[71–73] This renders it highly effective in diagnosing disorders such as Bernard-Soulier syndrome and Glanzmann's thrombasthenia (►**Fig. 1**), which involve alterations in specific platelet glycoproteins. Furthermore, flow cytometry is also able to diagnose qualitative or dysfunctional defects of various receptors, including GPIIbIIIa and GPIbalpha.

Moreover, it is worth noting that, under well-controlled pre-analytical conditions, flow cytometry presents the unique ability to detect and quantify microparticles and platelet aggregates. Microparticles[74] are small membrane-bound vesicles that are released from activated or apoptotic platelets and are increasingly recognized as important players in hemostasis and thrombosis. The ability to study these microparticles provides valuable insights into in vivo platelet function and pathophysiological processes.[75] Similarly, the detection and analysis of platelet aggregates can provide clues to platelet activation and aggregation status in vivo, contributing to a more comprehensive evaluation of platelet function or dysfunction. These additional observations not only broaden the diagnostic capabilities of flow cytometry but can also provide a more nuanced understanding of the complexities of platelet behavior in health and disease.[76]

Prior to analysis, single cells in suspension are labeled with specific fluorochrome-covered antibodies. Platelet activation can be induced by further addition of one or more agonists.[77] During analysis, the suspended single cells pass through a flow chamber with one or more laser beams, which activate the fluorophore at the excitation wavelength. Consequently, light is scattered from the cells as they pass through the light source in a fluid suspension according to the cell size and granularity. Multiple antibodies coupled to different fluorochromes can be used simultaneously. Also here, the use of microtiter plates has simplified the method and allowed higher throughput, the capacity to run more samples, and the ability to use more agonist concentrations simultaneously. In comparison to LTA, flow cytometry requires less blood volume and does not necessarily need PRP preparation.[78,79] Additionally, it is less sensitive to platelet count and therefore even

platelets from thrombocytopenic patients can be analyzed.[80] However, sample preparation remains labor-intensive and requires skilled personnel. Additionally, it needs technically advanced instruments and involves many manual steps. Thus, it is time consuming, especially when investigating different agonists and concentrations. Despite these challenges, flow cytometry is a promising technique for diagnosing well-characterized platelet disorders and may be performed before or complementary to LTA.[81–85]

The mepacrine assay is another potentially useful flow cytometry assay that enables evaluation of the secretion and incorporation capacities of platelets. It works by quantifying platelet fluorescence before and after stimulation. However, like many flow cytometry assays, it is affected by a lack of standardization, limiting its comparability and reproducibility across different laboratories. Moreover, while the mepacrine assay and lumi-aggregometry both contribute valuable insights, neither method can definitely distinguish between storage pool deficiency and primary secretion defects on their own. Storage pool deficiency is a condition where there are insufficient granules within the platelets, whereas primary secretion defects refer to impaired release of these granules upon platelet activation. The ability to differentiate between these conditions is crucial in the precise diagnosis and treatment of dense granule disorders. Therefore, a combined approach utilizing both the mepacrine assay and lumi-aggregometry has been proposed to better characterize dense granules disorders.[86]

Platelet Imaging Techniques

Platelet imaging techniques provide valuable information on platelet structure, function, and abnormalities, aiding in the diagnosis of various platelet disorders. Traditional methods include the use of dried blood smears, examined under light microscopy, which allow for the assessment of platelet count, size, and morphology. This simple and widely used technique can often give a first hint toward potential disorders, such as thrombocytopenia, thrombocytosis, or abnormal platelet morphology.

Fluorescence microscopy and other sophisticated imaging approaches are available in vivo and in vitro for further differentiation of functional and/or histological time-independent analysis of platelets.[87–96] Fluorescence microscopy has advanced our understanding of platelet biology by providing real-time visualization of intracellular processes. Fluorescent markers bind to specific platelet components or molecules, emitting light when excited, thus enabling the visualization of these components under a fluorescence microscope. This technique can illustrate various dynamic processes, such as platelet activation, adhesion, aggregation, and granule release.[97,98]

Additionally, confocal fluorescence microscopy, a more advanced technique, allows for the construction of three-dimensional images of platelets by taking multiple, thin, two-dimensional "slices" of the sample, further enhancing the detail and depth of platelet imaging.[99] Similarly, electron microscopy, including scanning electron microscopy (SEM) and transmission electron microscopy (TEM), can provide high-resolution images of platelet ultrastructure.[100]

These imaging techniques, combined with other diagnostic tools, offer a comprehensive approach to studying platelets, contributing to the diagnosis, understanding, and management of platelet disorders.

Platelet Function Analyzer—In Vitro Bleeding Time

One widely used point-of-care device represents the Siemens PFA instrument, a time-honored near-patient platelet function analyzer that employs high-shear citrated whole blood flow to simulate in vivo platelet aggregation and adhesion.[101–103] This instrument evaluates the ability of platelets to occlude a microscopic aperture composed of collagen-ADP (CADP)-or collagen-epinephrine (CEPI)-impregnated membrane mounted at the end of a capillary tube.[104] Platelet function is thus evaluated based on the closure time (CT, in seconds) needed to obstruct the membrane aperture by aggregated platelets. A prolonged CT may indicate platelet dysfunction or the presence of antiplatelet drugs. However, it is important to note that this instrument lacks sensitivity for certain conditions. For instance, while it is sensitive enough to detect severe platelet function disorders such as Bernard-Soulier syndrome, Glanzmann's thrombasthenia, and most forms of severe von Willebrand disease, it only demonstrates moderate responsivity toward milder platelets disorders, including secretory defects and storage pool disease.[104–106]

A controversial option to clinically verify bleeding disturbances is the bleeding time based on a standardized minor incision and timing of the cessation of bleeding.[107]

Viscoelastic Methods

Viscoelastic methods,[108] such as thrombelastography[109] (TEG 5000), rotational thromb(o)elastometry (ROTEM devices), ClotPro, and automated instruments using ultrasound-based sonorheometry[110] (e.g., 6S, Quantra) are technologies in diagnosing and managing hemorrhagic diatheses potentially associated with plasmatic and/or platelet disorders and additional effects based on erythrocytes and leucocytes, respectively, leading to bleeding or thromboembolic events.[111] These technologies provide a holistic, real-time depiction of the coagulation process, including clot formation, stabilization, and fibrinolysis, by examining the viscoelastic properties of the developing clot based on rheometry.[112,113]

TEG 5000, ROTEM, and ClotPro work on similar principles. An oscillating pin is placed in a whole blood sample, and the changes in resistance as the blood clots and subsequently lyses are recorded.[108] This reveals critical information about clot kinetics, strength, stability, and fibrinolysis, thereby helping to identify the share of platelet shortage (potentially associated with platelet dysfunctions). Special reagents are needed to identify and monitor antiplatelet therapy (i.e., platelet mapping) using thrombelastography or sonorheometry.[114]

However, all these methods require specific expertise for interpretation of results and may not detect specific platelet function abnormalities as effectively as other specialized platelet function tests in acute complex clinical scenarios.

Molecular Genetics and New Scientific Approaches

Over the last decade, high-throughput sequencing has revolutionized the genetic diagnosis of human diseases using targeted gene sequencing (TGS), whole exome sequencing (WES), and whole-genome sequencing (WGS).[115,116] The costs of these technologies have constantly declined, and these techniques are meanwhile widely available in both research and clinical practice, becoming a sort of gold standard for identifying monogenic diseases, such as in patients with inborn bleeding diseases.[21,117–119] Catalogs of clinical features associated with specific genes are available in online databases such as *www.omim.org* and *www.genecards.org*.[13] Additionally, the SSC-GinTH has curated a list of genes associated with bleeding disorders to create a recommended gene panel for clinical use, which is available via *clinicalgenome.org*.[120] One of the advantages of this method is that the sample required for testing is generally EDTA whole blood. Some laboratories are able to accept as little as 1 mL of whole blood or buccal swabs to perform the sequencing, making also pediatric patients suitable to test when clinically justifiable. However, genetic testing still suffers from limitations, such as lack of accessibility, high costs, and sometimes difficulties in assigning pathogenicity to newly identified variants, in addition to ethical debates surrounding its use.[121,122] Moreover, the interpretation of variants of uncertain significance (VUS) remains a challenging aspect of genetic testing. Without complementary first-line functional tests, determining the pathogenicity or clinical relevance of these variants can be complex and uncertain. These functional assays can provide important phenotypic data that, when correlated with the genotypic findings, can help clarify the role of identified genetic variants and inform clinical management. However, the interpretation of VUS without such data would rely heavily on available literature, population databases, and predictive computational models, which may not always provide conclusive evidence of pathogenicity. Hence, careful interpretation and communication of these results is essential to avoid misinterpretation and misdiagnosis.[123–125]

Further research approaches are developed and implemented to assess platelet function and properties using proteomics, lipidomics, and transcriptomics, revealing that platelets no longer represent a homogeneous cell population as previously thought. Instead, they constitute a heterogeneous, interactive population with distinct subgroups that either protect against or contribute to disease processes.[126]

Is There Life in the Old Light Transmission Aggregometry Yet?[a]

The study of platelet dysfunction is inherently complex due to the heterogeneity of the underlying pathophysiology.[127] Therefore, no single method has yet been identified as the definitive and universally simple diagnostic for platelet dysfunctions. While LTA has established itself as gold standard due to its ability to detect a wide range of inherited, acquired, and drug-induced platelet defects, it is not without limitations and concerns, many of which are currently being addressed through technical improvements and implementations. Among the ongoing debates is the question of whether it is more appropriate to use PRP or whole blood for analysis. PRP, as a non-physiological matrix, has been criticized for its inability to reflect interactions with the endothelium or incorporate any sheer stress, both of which are known to be significant in vivo processes.[106] In contrast, whole blood analysis simulates platelet aggregation under more physiological conditions, as contributions from other blood components are included. In addition, whole blood methods use a small amount of blood in which all subpopulations of platelets are present, allowing rapid analysis of platelet function without the need for prior activation or manipulation of the sample. However, the different analyzers often lack flexibility, have limited availability, and employ different technologies, often yielding disparate interpretations. Additionally, their performance in diagnosing constitutional platelet pathologies is poorly documented. Therefore, further research is urgently needed to evaluate their potential role in diagnosing IPDs.

Until now, results obtained by LTA should be supplemented by other analytical (e.g., flow cytometry analysis for identification and quantification of specific platelet components, assessment of platelet secretion, and specific assays for platelet compounds) or genetic tests, depending on availability.

Flow cytometry has the advantage that of requiring only small volumes of blood, a particularly important factor when evaluating pediatric patients or those with high hematocrit and low proportion of plasma.[128] It also has potential applications in thrombocytopenic patients.[34] Unlike LTA, which measures overall platelet aggregation and is highly sensitive to nonsteroidal anti-inflammatory drugs (NSAIDs) due to their inhibitory effect on platelet function,[129] flow cytometry may be less affected by NSAIDs. This is especially the case when ADP, cross-linked collagen-related peptide (CRP-XL), and TRAP-6 are used as agonists. However, the extent to which NSAIDs impact flow cytometry assays can depend on several factors, including the specific parameters of the assay and individual patient characteristics. Therefore, although there are scenarios where flow cytometry may appear less sensitive to NSAIDs than LTA, the impact of these drugs can vary and should be considered when interpreting results from both methods.[130] It is also worth noting that flow cytometry is not able to study aspects and signaling induced by contacts between platelets and platelets. Moreover, several of the proposed protocols for flow cytometry have yet to be validated by studies and lack standardization.[131] Therefore, it is essential to ensure that NSAIDs were definitely stopped before performing diagnostic tests for IPDs.

Since the implementation of next generation sequencing (NGS) in 2009, a rapid analysis of genes previously implicated in IPDs or those known to have a key role in platelets has become available.[132,133] This also extends to novel genes not previously implicated in platelet dysfunction. However, despite significant advances in our understanding of the molecular basics of IPDs, molecular genetic approaches still do not fulfill all the requirements necessary to elucidate the

[a] There is life in the old dog yet—Totgesagte leben länger

underlying genetic variance in every pathological case. A considerable proportion of symptomatic patients cannot be diagnosed and, in some instances, the pathogenic mechanisms of certain variants cannot be confirmed or correctly interpreted at present.[134–136] Consequently, a precise description of the functional phenotype using the methods mentioned in this article represents a crucial prerequisite for establishing a valid phenotype–genotype correlation.

Glanzmann's thrombasthenia accounts here for a prominent example: This IPD is characterized by a normal platelet count, prolonged bleeding time, abnormal clot retraction, and defective platelet aggregation in response to multiple physiologic agonists. The defect may be caused by quantitative or qualitative abnormalities of the platelet integrin receptor, αIIbβ3, the primary player in platelet function. While Glanzmann's thrombasthenia shows a straightforward pattern of aggregation using LTA, describing the pathology at the genetic molecular level reveals a wide variety of polymorphisms,[137–139] leading to the observed pathology.[42]

To exemplify, 45 unrelated patients who were unequivocally diagnosed with Glanzmann's thrombasthenia based on their phenotype were subjected for the genetic analysis in αIIb and β3 genes. Both of these proteins form a heterodimer complex (αIIbβ3) that undergoes final processing and is then transported to the platelet surface. In nine of these patients (20%), no causative gene alterations were identified in both the *ITGA2B* and *ITGB3* genes, respectively.[140]

This suggests that any defect in the regulatory proteins involved in integrin activation or its binding sites on αIIbβ3, as well as any defect in regulatory elements affecting the transcription or post-translational modifications of αIIb and β3, could potentially affect the integrin activation process, leading to the condition of Glanzmann's thrombasthenia.[141]

Additionally, there are also other pathologies, as for example the sticky platelet syndrome,[142–144] which is considered as an autosomal dominant disorder associated with arterial and venous thromboembolic events where the precise underlying genetic defects remain unidentified,[145–147] or where certain subpopulations of pro-coagulant platelets may potentially be missing.[148,149]

Taken together, neither LTA nor genetic testing alone can provide a comprehensive diagnosis for patients with IPDs. The combination of functional and genetic testing is the key for accurate phenotype–genotype characterizations. From our point of view, there are two potential future scenarios for the utilization of LTA in diagnosing IPDs:

1. Improvements in automation, standardization, and usability are likely to render LTA available outside of specialized centers in the next few years. This is particularly important since whole blood drawn for LTA platelet function testing expires in 4 hours or less. This short sample viability requires that a specialized laboratory be within 2 hours away to ensure that sufficient time remains to perform the analysis. However, many clinics and hospitals are not fortunate to be in geographic proximity to a specialized coagulation laboratory that performs this test. When the issues explicated in this article will be properly addressed and solved by future inventions and strict guidelines, LTA will very likely remain the first-line functional platelet test and will defend its position as gold standard.
2. Given that LTA still has many limitations and results remain difficult to interpret in a significant proportion of cases, its place in the workflow of diagnosing IPDs may be revised. As further research is done on platelet disorders and on the identification of the causal variants associated with the disease, NGS may soon be fully integrated into the diagnostic setting. The sample required for genetic testing is generally EDTA whole blood and is very stable when shipped across countries. From its present use as a first-line screening test, LTA could move on to become a second-line functional test for confirmation of genetic variations identified by high-throughput sequencing techniques.

Conclusions

Light transmission aggregometry has been the gold standard method for detecting platelet defects and assessing platelet function for many years. However, with technological advancements and the availability of alternative tests, the clinical utility of LTA has evolved and may vary depending on the particular clinical scenarios and laboratory resources.

In recent years, other platelet function tests, such as flow cytometry, impedance aggregometry, and PFA, have emerged as potential alternatives to classic LTA. These newer methods offer advantages such as a faster turnaround time, lower variability, and user-friendliness. Yet, they also have their own limitations and may not be universally applicable across all clinical settings. Consequently, no alternate approach has yet emerged to effectively replace LTA in diagnosing IPDs.

The decision to use LTA or other platelet function tests depends on a variety of factors, including the clinical context, availability of resources, and expertise of the laboratory. In some cases, LTA may remain the first-line test for identifying platelet defects, particularly in specialized laboratories with expertise in performing and interpreting the results. In other cases, alternative tests might be preferred due to their convenience, speed, and reproducibility.

Therefore, it is important to consult with a qualified healthcare professional or a clinical laboratory expert to determine the most appropriate platelet function testing method for a specific patient or clinical scenario, as it may vary depending on the individual circumstances.

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgment

We thank Erica L. Weiss for her meticulous refinement of the manuscript's language.

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