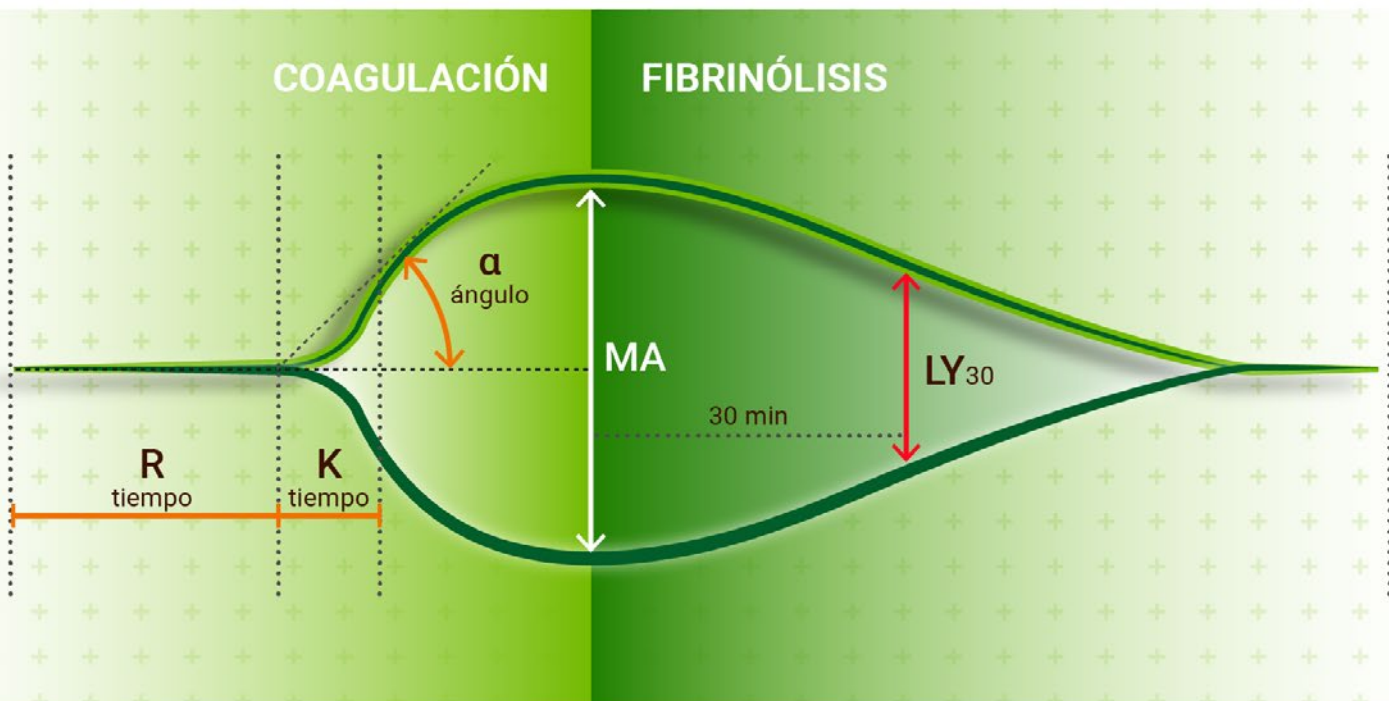


Hemostasia y Trombosis

REVISTA IBEROAMERICANA DE TROMBOSIS Y HEMOSTASIA

Volume 2 · Number 2 · April-June 2025



SETH

SOCIEDAD ESPAÑOLA DE
TROMBOSIS Y HEMOSTASIA



Grupo Cooperativo
Latinoamericano de
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Editorial

Editorial

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In this third issue of the journal, we continue our editorial commitment to offer rigorous, updated, and clinically relevant content in the field of haemostasis and thrombosis. The constant evolution of the discipline, with continuous therapeutic and technological innovation, leads us to the need for spaces for scientific reflection that allow us to share experiences, contrast results, and foster evidence-based healthcare improvement. This scientific reflection must be, due to the progressive complexity of the field, multidisciplinary and as broad as possible. Progress in haemostasis and thrombosis cannot be imagined without this transversality. In this regard, we want to highlight the publication in this issue of the first part of a multidisciplinary consensus document on viscoelastic tests, which, with their option of use as a point-of-care coagulation tool, have become an enormously useful instrument in the management of patients with acute bleeding. This consensus has been drafted with the participation of experts from three societies involved in the use and development of viscoelastic test applications: the Spanish Society of Anaesthesiology, Resuscitation and Pain Therapy (SEDAR), the Spanish Society of Laboratory Medicine (Semedlab), and the Spanish Society of Thrombosis and Haemostasis (SETH). The first part of the consensus, which is published in this issue, addresses the review of the technical foundations of available devices for viscoelastic tests, the clinical scenarios where their application is best established, analytical quality protocols, health care implementation models,

and the financial impact of their use in relation to the saving of blood products and shorter lengths of stay. The vision of these experts from the areas of anaesthesiology, haematology, and laboratory allows us to know first-hand the latest in this type of analyser.

Continuing in the field of the haemostasis laboratory, this issue presents a multicentre study conducted in 3 laboratories in the city of Buenos Aires (Argentina), which analyses the interference of lupus anticoagulant in the analytical measurement of the activity of factors VIII, IX, and XI. To resolve this interference, some methods have been proposed that unfortunately cannot be performed in most laboratories, and their use is limited to a few specialised centres. The work proposes the use of an activated partial thromboplastin time reagent that uses a silica-based activator, with a high concentration of phospholipids, which allows, in the presence of lupus anticoagulant, to obtain more reliable values of the factors than coagulative and chromogenic methods. The results show that the proposed method can avoid false diagnoses of factor deficiency, improving diagnostic precision in patients with acquired or autoimmune coagulopathies.

In the field of bleeding disorders, several contributions focused on congenital bleeding coagulopathies are presented. First, it is worth highlighting a very interesting review on the current treatment of Von Willebrand disease, focused on the value of performing prophylaxis with blood products. After analysing the available evidence, the authors conclude that prophylaxis in patients with a past medical history of severe and frequent

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Date of reception: 01-07-2025
Date of acceptance: 10-07-2025
DOI: 10.24875/RHT.M25000048

Available online: 18-09-2025
Hemost Trombos (Eng). 2025;2(2):41-42
www.revistahemostasiaytrombosis.com

bleeding is superior to on-demand treatment, and that it should be applied regardless of the type of Von Willebrand disease, age, and sex.

Additionally, this issue includes 2 case reports, both focused on the treatment of haemophilia A with emicizumab. The first is a detailed clinical case of a young patient with severe haemophilia A without inhibitors, in whom the progressive decrease in the efficacy of extended half-life factor VIII led to a change of drug to achieve adequate disease control. Case report #2 refers

to 2 paediatric patients with haemophilia A on emicizumab who developed anti-drug antibodies, analysing the analytical evolution, the impact on plasma concentrations of emicizumab, and the measures adopted to maintain prophylaxis.

Overall, this issue of *Hemostasia y Trombosis – Revista Iberoamericana de Trombosis y Hemostasia* offers a diverse and updated overview of some important challenges and advances in the practice of clinical and laboratory haemostasis.

Multidisciplinary perspective on the use of viscoelastic tests in clinical practice (part 1)

Perspectiva multidisciplinaria del uso de test viscoelásticos en la práctica clínica (parte 1)

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Abstract

Viscoelastic tests (VET) or point-of-care coagulation (POCC) devices are key tools in hemorrhage management, especially in surgery, but their use is expanding to other fields such as obstetrics, ECMO (extracorporeal membrane oxygenation) patients, and burn victims. While their role in cardiac surgery, polytrauma, and liver transplantation is more established, evidence regarding their impact on mortality remains controversial. One of the main limitations is the lack of result validation, as VETs are not comparable to standard coagulation tests, showing significant variability (up to 83% in ROTEM®). Additionally, quality control is not always managed by the laboratory, which may affect reliability. Anesthesiology, laboratory medicine, and hematology are involved in their interpretation. This document, supported by scientific societies from these specialties, aims to provide a comprehensive perspective on their functionality, scientific evidence, implementation, quality control, and potential economic benefits.

Keywords: Viscoelastic tests. Thromboelastography. Thromboelastometry. Consensus.

Resumen

Los test viscoelásticos (TVE) o point of care de coagulación (POCC) son herramientas clave en el manejo de la hemorragia, especialmente en cirugía, pero su uso se expande a otros ámbitos como obstetricia, pacientes en ECMO (oxigenación por membrana extracorpórea) y quemados. Aunque su papel en cirugía cardíaca, politraumatizados y trasplante hepático está más consolidado, la evidencia sobre su impacto en la mortalidad sigue siendo controvertida. Una de las principales limitaciones es la falta de validación de resultados, ya que los TVE no son comparables con los test estándar de coagulación, y muestran una variabilidad significativa (hasta el 83% en ROTEM®). Además, el control de calidad no siempre depende del laboratorio, lo que puede afectar su fiabilidad. Anestesiología, laboratorio y hematología participan en su interpretación. Este documento, respaldado por sus sociedades científicas, busca ofrecer una visión integral sobre su funcionamiento, evidencia, implementación, control de calidad y posibles beneficios económicos.

Palabras clave: Test viscoelásticos. Tromboelastografía. Tromboelastometría. Consenso.

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Date of reception: 21-02-2025

Date of acceptance: 13-05-2025

DOI: 10.24875/RHT.M25000050

Available online: 18-09-2025

Hemost Trombos (Eng). 2025;2(2):43-59

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Introduction

Viscoelastic tests (VETs) or point-of-care coagulation (POCC) tests have become important tools in hemorrhage management in various settings.

While surgical patients have been the primary beneficiaries of VET-guided management, an increasing number of non-surgical scenarios can also benefit from these devices.

In some settings, the use of VETs appears well-established. Specifically, cardiac surgery, polytrauma care, and liver transplantation are procedures where VETs have been firmly established as guides for hemostatic correction therapy. There are other specialties where these POCC devices are not yet established, but scientific literature opens new avenues for their use, such as in obstetrics, burns, patients on ECMO (extracorporeal membrane oxygenation), etc.

The reality is that even in settings where VETs seem most consolidated, the scientific evidence is controversial, and robust conclusions regarding reductions in mortality or improvements in clinical outcomes remain elusive in patients whose hemostasis has been guided by objectives derived from POCC device results.

There is an apparent lack of result validation, partly because VETs are not comparable to standard coagulation tests. Although the coefficient of variation (a measure of precision and internal quality control for an assay) is 5% for standard laboratory tests, it reaches 39% for thromboelastography (TEG) and up to 83% for thromboelastometry (ROTEM®).

Quality control of VETs in many centers is external to the laboratory service and is performed by the regular users of the device, but this fact can also reduce the reliability of the results.

The clinical use and interpretation of VET results involves collaboration among various medical specialties, specifically anesthesiology, laboratory, and hematology. Scientific societies from these three specialties have collaborated on this document to present a global perspective on these devices and to convey the most up-to-date information on their basic operating principles, scientific evidence for their use in various clinical scenarios, different implementation models, quality control, and cost savings, among other aspects.

Purpose of the document

- To jointly present, on behalf of the three scientific societies, the clinical relevance and utility of POCCs

as a hemostatic management measure in bleeding patients.

- To reach a consensus on the best way to perform these tests, so that we can reduce their analytical variation, expressed as the coefficient of variation in their results, and thus provide robustness to them.
- To examine the economic suitability of using these devices in relation to the savings in blood products and earlier control of hemorrhagic events.

Document recipients

Physicians from different specialties who manage or wish to manage these VETs as part of their therapeutic arsenal, i.e., therapeutic and diagnostic.

Description of available VETs on the market. Operating principles

Fundamentals of VETs

These devices offer a more physiologically accurate assessment of coagulation than conventional laboratory tests. These tests provide dynamic and global information on the viscoelastic properties of blood by integrating platelets, coagulation cascade components, and cellular blood elements. Basically, the assays involve inducing *in vitro* coagulation of the patient's sample with different reagents that activate the intrinsic or extrinsic pathway and reflect the clot characteristics. There are various analyzers for performing viscoelastic tests, which, through different technologies, offer results in the form of graphs and parameters with numerical data informing about clot quality (Table 1).

Although the available analyzers could be considered to aim for the same objective, they present differences among themselves. Table 2 illustrates the main advantages and disadvantages of each.

Thromboelastography TEG® 5000

The principle of the rotational thromboelastography technique of the TEG® 5000 (Haemonetics) consists of inducing *in vitro* coagulation of a blood sample (360 µL) from the patient in a small cuvette that rotates with uniform and constant motion. The disposable cuvette, maintained at 37°C, rotates alternately (4° 75' every 5 s) around a small fixed shaft or piston. As blood clots, resistance from the clot increases, and these variations are recorded by an electromagnetic transducer, which converts them into an electrical signal that

Table 1. Point-of-care coagulation devices available on the market

Analyzer	Company	Technology	Presentation
TEG® 5000	Haemonetics	Rotational thromboelastography	Independent liquid reagents
ROTEM® Delta	Instrumentation laboratory (Werfen)	Rotational thromboelastometry	Independent liquid reagents
ROTEM® Sigma	Instrumentation laboratory (Werfen)	Rotational thromboelastometry	Cartridge tests
ClotPro®	Enicor GmbH (Haemonetics)	Rotational thromboelastometry	Lyophilized reagents preloaded in tips
TEG® 6S	Haemonetics	Harmonic resonance	Cartridge tests
Quantra®	Hemosonics (Stago)	Sonorheometry (SEER)	Cartridge tests
Sonoclot®	Sienco Inc.	Microviscometry	Independent liquid reagents

Table 2. Advantages and disadvantages of different devices

Test	Advantages	Disadvantages
Individual Tests ROTEM® Delta ClotPro® Sonoclot®	Smaller sample volume Valid for low sample volume (paediatric) Allows selection of test based on algorithm; more affordable	Greater ariability due to manual sample pipetting Requires more user training
Cartridge Tests ROTEM® Sigma TEG® 6S Quantra®	Easier sample processing Less need for expert personnel Lower pre-analytical ariability	Larger sample volume (except TEG® 6S) Not valid for low sample volume (paediatric); more expensive

the analyzer's software translates into a graph and numerical results (Fig. 1). The recorded variations depend on both the kinetics and the characteristics of the clot¹⁻³.

The analyzer has two independent channels and liquid reagents for performing the tests. The available parameters and tests are found in tables 3, 4, and 5.

In April 2023, a change was made to the FIBTEM C reagent contained in ROTEM® Sigma Complete and Sigma Complete + Heparin cartridges. The formulation was improved with the addition of tirofiban to the already existing cytochalasin D, both platelet inhibitors, thereby achieving almost total elimination of platelets in the FIBTEM C test. The aim of the new formulation was to address a problem with samples having high platelet levels.

In 2024, a Welsh working group observed a decrease in FIBTEM C A5 cutoff values (1-2 mm) in obstetric patients compared to previous cartridge batches (IVDD) following the test improvement with the addition of tirofiban. This means that clinical decision algorithms might require adjustment to avoid inappropriate (additional) fibrinogen concentrate transfusions. However,

evidence only exists at a single center, and it remains to be seen whether this modification also affects other scenarios (cardiac surgery, liver transplantation, or traumatology).

Rotational thromboelastometry (ROTEM® Delta)

Rotational thromboelastometry ROTEM® has a very similar principle to TEG. Its technology is based on the disposable cuvette, which is maintained at 37°C and on which the patient's citrated whole blood (330 µL) is deposited, remaining fixed, while the vertical shaft or piston introduced into the sample oscillates (4° 75' every 6 s). The movement is detected by an optical system located at the upper end, whose data are transferred and processed by the analyzer's specific software⁴. When the sample coagulates, the resistance to the piston's rotation changes, reducing the rotation amplitude of the shaft, and these data are recorded and interpreted by the analyzer, transforming them into a graph with their values, which depend on the kinetics and characteristics of the clot (Fig. 2).

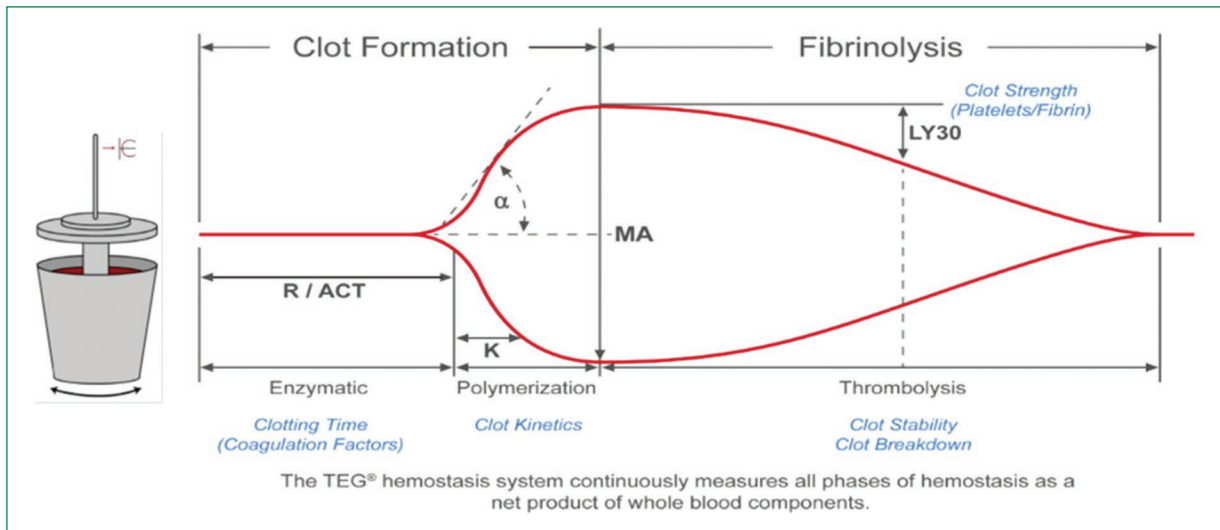


Figure 1. Phases and parameters of clot formation in the TEG system (courtesy of Ferrer, Haemonetics Corp.¹).

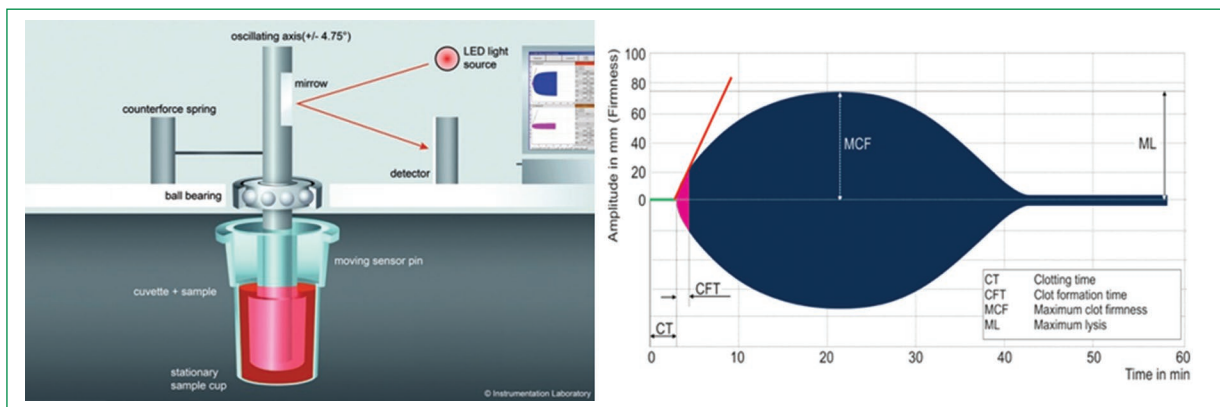


Figure 2. Diagram of the ROTEM system's operation (courtesy of Werfen⁴).

The Delta model has 4 independent channels and liquid reagents for performing five different tests that can be processed independently and according to algorithms or indications^{2,3,5}. Parameters and tests are available in tables 3, 4, and 5.

Rotational thromboelastometry (ROTEM® Sigma)

This system is primarily designed for point-of-care testing (POCT). It is based on cartridge technology, is a closed system, easy to use, and fully automated; no pipetting or test preparation is necessary. The tube containing the citrated whole blood sample is directly punctured into the cartridge, which is then inserted into the analyzer (Fig. 3). Each cartridge contains

chambers/wells with the necessary reagents to perform the different tests. Results are expressed in graphs and numerical data similar to the ROTEM® Delta.

Parameters and available tests with their description can be found in tables 3, 4, and 5.

ClotPro® system thromboelastometry

The ClotPro® system^{1,6} is a 6-channel viscoelastometry analyzer that uses elastic motion thrombelastography, an improved next-generation viscoelastometry technique. Similar to conventional rotational thromboelastometry systems, the cup and pin surfaces experience a relative movement of about 5° driven by an elastic element. In the ClotPro® analyzer, the cup rotates while the pin remains stationary. The cup's rotation

Table 3. Parameters of different viscoelastic test analyzers

Parameter description	TEG® 5000	ROTEM® Delta & Sigma	ClotPro® System	TEG® 6S	Quantra®	Description
Reaction/Clotting time	R/ACT (s)	CT (s)	CT (s)	R/ACT (s)	CT (s)	Parameters for assessing clot formation (coagulation factors and anticoagulants)
Clot formation time	K (s)	CFT (s)	CFT (s)	K (mm)	-	
Clotting time with heparinase	-	-	-	-	CTH (s)	
Clotting time ratio	-	-	-	-	CTR	
Clot strength. Amplitude at various times (5, 10, and 20 minutes)	A10 (mm)	A5, A10, A20	A5, A10, A20	A10 (mm)	-	Parameters for assessing clot firmness
Fibrinogen contribution to clot strength	-	-	-	-	FCS (hPa)	
Platelet contribution to clot strength	-	-	-	-	PCS (hPa)	
Maximum clot firmness	MA (mm)	MCF (mm)	MCF (mm)	MA (mm)	CS (hPa)	
Clot lysis stability. Lysis at 30 minutes	LY30 (%)	LY30 (mm)	LY30 (mm)	LY30 (mm)	-	Parameters for assessing clot stability and/or fibrinolysis
Clot stability. Maximum lysis	ML	ML	ML	-	CSL (%)	

A5: clot amplitude at 5 minutes; A10: amplitude at 10 minutes; A20: amplitude at 20 minutes; CFT: clotting firmness time; CS: clot stiffness; CT: clotting time; LY: lysis; FCS: fibrinogen contribution to clot stiffness; CSL: clot stability to lysis; K (K-time): clot formation time; MA: maximum clot firmness in TEG; MCF: maximum clot firmness in ROTEM; PCS: platelet contribution to clot stiffness; R/ACT: activated clotting time result.

Table 4. Cartridges and tests available on TEG® 6S, ROTEM® Sigma, and Quantra®

Analyzer	Cartridge	Test	Description
ROTEM® Sigma	Rotem Sigma Complete	EXTEM	Rapid assessment of clot formation, fibrin polymerization, and fibrinolysis via extrinsic and intrinsic pathways
		INTEM	Qualitative evaluation of fibrinogen status
		FIBTEM	Differentiates between hyperfibrinolysis and platelet-mediated clot retraction/FXIII deficiency
		APTEM	Particularly suitable for patients requiring fibrinolysis diagnosis
	Rotem Sigma Complete+Hep	EXTEM	Rapid assessment of clot formation and fibrin polymerization via extrinsic and intrinsic pathways
		INTEM	Qualitative evaluation of fibrinogen status
		FIBTEM	Differentiates between factor deficiency and heparin effect
		HEPTEM	Recommended for patients on therapeutic heparin and endogenous heparin (heparinoids)
TEG® 6S	Global Hemostasis Cartridge	Kaolin test Kaolin TEG with Heparinase RapidTEG™ Functional Fibrinogen TEG	Provides a global assessment of patient hemostasis Qualitative evaluation of fibrinogen status Differentiates between factor deficiency and heparin effect
	PlateletMapping® Cartridge	PlateletMapping®	Assesses platelet activity by studying its aggregation capacity
Quantra®	QPlus Cartridge		Evaluates clot formation, the contribution of fibrinogen and platelets to clot firmness, and the influence of heparin Recommended for cardiovascular and orthopedic surgery
	QStat Cartridge		Includes tests to evaluate clot characteristics and lysis via intrinsic and extrinsic pathways, and includes a test with tranexamic acid Indicated for intensive care, traumatology, and liver transplantation

Table 5. Tests available for TEG® 5000, ROTEM® Delta, and ClotPro®

TEG® 5000	ROTEM® Delta & Sigma	ClotPro® system	Description
-	Extem	Ex-Test	Rapid coagulation assessment, activating the extrinsic pathway with recombinant tissue factor
Kaolin TEG	Intem	In-Test	Evaluates the intrinsic pathway by activating it with Kaolin or ellagic acid
Functional Fibrinogen TEG	FIBTEM	Fib-Test	Evaluates the contribution of fibrin and platelets to clot firmness. Eliminates platelet participation through a GPIIb/IIIa antagonist inhibitor and/or cytochalasin D
Kaolin TEG with Heparinase	Heptem	Hi-Test	Evaluates the presence of heparin or synthetic heparinoids in the sample by the action of heparinase
-	Aptem	Ap-Test	Evaluates fibrinolysis using aprotinin as a direct plasmin antagonist
RapidTEG™	-	-	Assay to rapidly evaluate coagulation properties by activating the intrinsic pathway with Kaolin and the extrinsic pathway with tissue factor
-	-	RVV-Test	Screening test to detect direct factor Xa anticoagulants. Uses Russell's viper venom as a direct FX activator
-	-	ECA-Test	Screening test to detect direct factor II (thrombin) anticoagulants. Uses ecarin as a prothrombin activator
-	-	TPA-Test	Allows assessment of patient antifibrinolytic therapy. For this, it induces fibrinolysis using recombinant tissue plasminogen activator
TEG PlateletMapping®	-	-	Evaluates platelet inhibition/aggregation



Figure 3. Example of a cartridge for the ROTEM® Sigma (courtesy of Werfen®).

is detected by an electronic sensor sensitive to changes in rotation. During clot formation, the movement between the cup and the pin decreases as clot resistance increases. This data is recorded by the analyzer, which interprets it in the form of graphs and numerical results (Fig. 4).

Reagents for each assay come lyophilized and preloaded in pipette tips. The citrated blood sample is pipetted with these tips (340 µL), allowing for simple and safe mixing with the reagent. The analyzer has six independent channels and nine different tests whose reagents are preloaded in pipette tips for independent performance.

Currently, only the ClotPro® system has specific screening tests for the detection of direct oral anticoagulants (DOACs) that are antagonists of coagulation factor X (FX) (apixaban, rivaroxaban, edoxaban) and thrombin (FII) (dabigatran)^(a,b). They show high sensitivity and specificity for detecting DOACs, which is very useful in emergency situations.^{7,8} The available parameters and tests with their descriptions can be found in tables 3, 4, and 5.

TEG® 6S thromboelastogram

The TEG® 6S system (Haemonetics)¹ uniquely employs harmonic resonance technology, assessing the physical properties of the clot using sound waves, such that clot formation is accompanied by changes in resonance frequency. To measure clot strength with the resonance method, the sample is exposed to a fixed vibration frequency. As the sample transitions from a liquid state to a gel-like consistency during coagulation, the elastic modulus and resonance frequency increase. Resonance frequency increases over time and with clot firmness. This analyzer uses a cartridge system containing the necessary reagents for the tests; it is a closed and fully

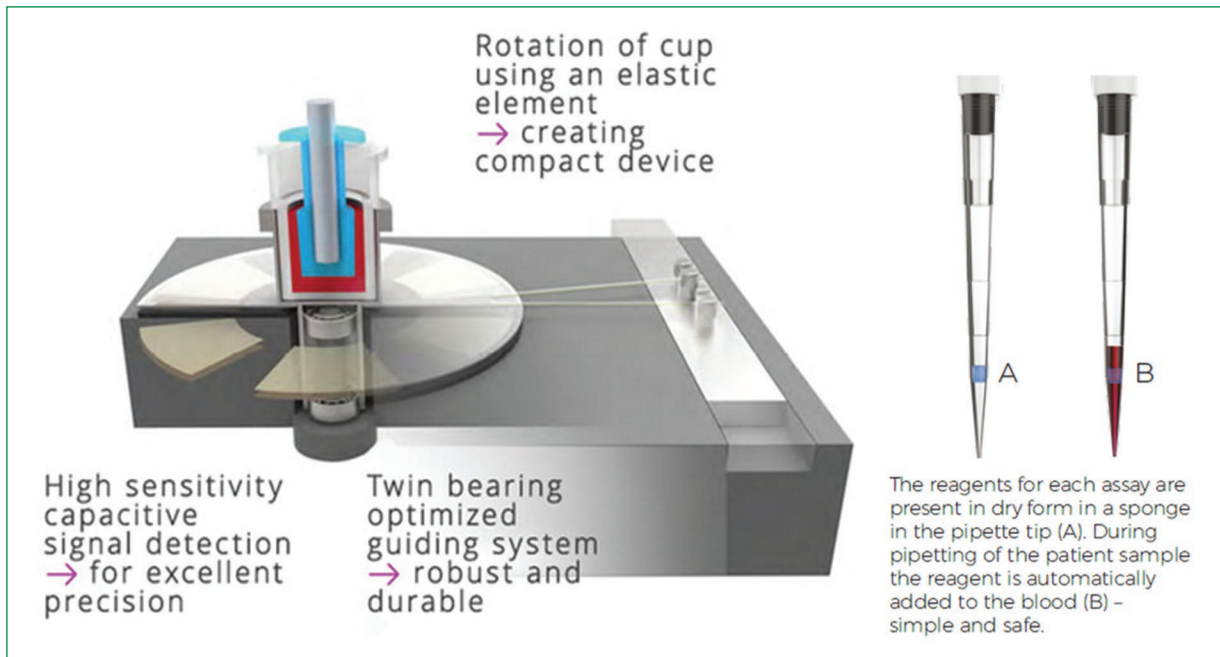


Figure 4. Diagram of the ClotPro® system's operation and tips with preloaded reagent (courtesy of Ferrer, Haemonetics Corp.¹).

automated system. Four hundred μL of whole blood are directly pipetted into the cartridge. In the wells/chambers of the cartridge, the clot forms and is subjected to sound waves. The analyzer displays the results in the form of graphs and numerical values (Fig. 5).

The available parameters and tests with their descriptions can be found in tables 3, 4, and 5.

Quantra® system

The Quantra® system (Hemosonics) is based on a technology called SEER sonorheometry⁹⁻¹² (sonic estimation of elasticity via resonance). It uses ultrasound technology to measure changes in the viscoelasticity of the sample. This technology measures clot elasticity at time intervals using ultrasound, and the evolution of elasticity is represented in a graph expressed in hectopascals/minute (hPa/min). By measuring elasticity, the PCS parameter (platelet contribution to clot stiffness) is obtained, which is related to the number and function of platelets. This fully automated system is designed for ease of use in POCT; it uses a cartridge system, is easy to use, and fully automated. The tube with the citrated whole blood sample is directly punctured into the cartridge. The sample is subjected to ultrasound pulses that generate data interpreted by the analyzer and displayed in the form of dials and large numerals (Fig. 6).

Sonoclot® sienco

This analyzer is described as a microviscosimeter.¹³ The electronic components are sensitive to any resistance to the movement of the oscillating probe within the blood sample. The higher the fluid viscosity, the greater the output signal generated by the analyzer. The output signal is calibrated according to reference viscosity standards and is reported in normalized “clot signal” units. The analyzer displays a graph representing the different phases in clot formation, from coagulation activation, through fibrin formation and clot retraction, to the formation of a mature clot (Fig. 7).

Different tests are available for the Sonoclot® model that allow evaluation of clot formation, heparin anticoagulation control, and platelet function. Currently, this analyzer is not marketed in our country.

Analytical considerations and implementation modes of VETs

Pre-analytical phase

Suitable containers for these types of tests are tubes with 3.2% sodium citrate as an anticoagulant (blue cap). Although some analyzers can use whole blood without anticoagulant, this is not recommended due to the possibility of sample coagulation. To prevent

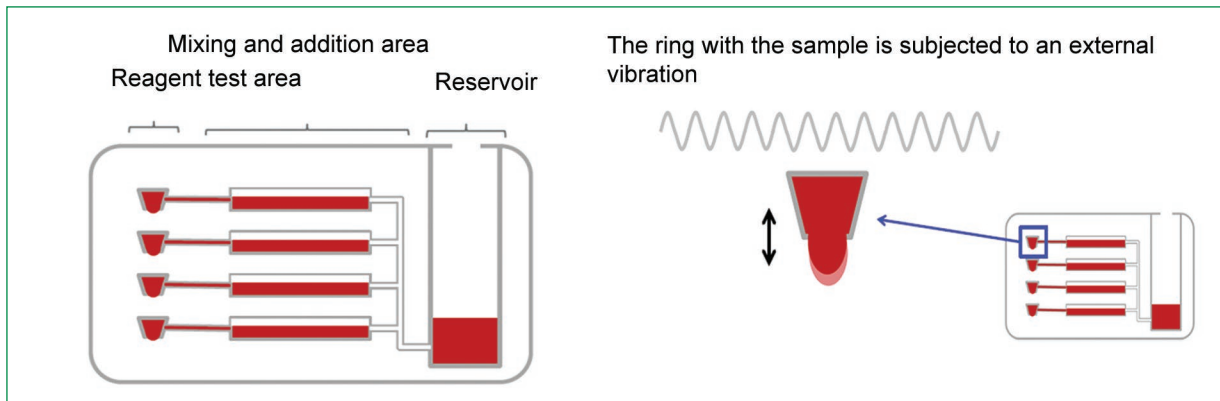


Figure 5. Diagram of the cards and operation of the TEG® 6S system (courtesy of Ferrer, Haemonetics Corp.¹⁾).

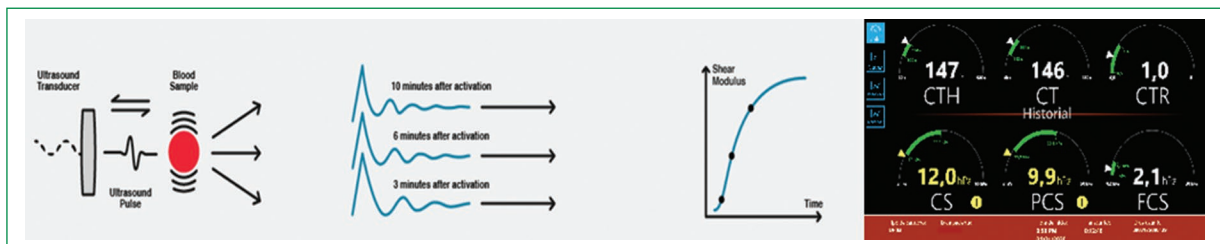


Figure 6. Diagram of sonorheometry technology (SEER, sonic estimation of elasticity via resonance) (courtesy of Biometa⁹).

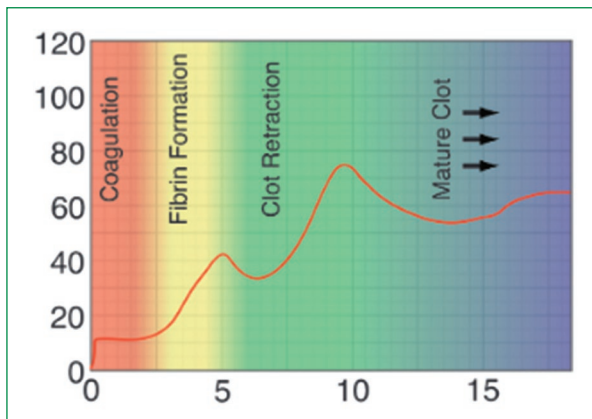


Figure 7. Hemostatic phases of the Sonoclot® Sienco (taken from Sienco Home¹³).

sample dilution by the anticoagulant, it is recommended that tubes be filled to their maximum capacity recommended by the manufacturer.

Once the sample has been drawn, the tube must be processed within 4 hours of collection. There are no recommendations for sample resting time. For ROTEM, sample processing is recommended between 30^{14,15} and

120 minutes¹⁶ after collection. Refrigeration or freezing is not advised. Other authors recommend that samples be analyzed immediately,¹⁷⁻¹⁹ which is consistent with the immediacy of clinical actions derived from the results.

Since these types of tests are sensitive to small variations, both analytical and biological, centers are recommended to take appropriate measures to reduce variation in their measurements.

Skill is required to detect subtle variations in results obtained with patient samples, but for this, errors made in sample collection, preparation, handling, and analysis that might mask these physiological variations must be minimized.

Despite this pre-analytical variability, some studies encourage users of viscoelastic tests to use evidence-based guidelines developed for these assays²⁰ to minimize sources of variation and, therefore, maximize the diagnostic utility of these methods.

PRE-ANALYTICAL RECOMMENDATIONS THAT SHOULD BE TAKEN INTO CONSIDERATION

– Avoid sample hemodilution from line drawing.

- Fill the citrate tube to the recommended level to prevent hemodilution by the liquid anticoagulant (citrate).
- Homogenize the drawn sample to prevent coagulation.
- Perform tests as soon as possible, whether performed as POCT or sent to the emergency laboratory.
- If determinations are requested from the emergency laboratory, it is recommended to establish a consensual protocol between departments to expedite the process.
- Reference ranges for each test should be evaluated and adapted to the reference population.

Quality control

Internal quality control is a procedure used to detect, reduce, and correct possible analytical deficiencies before issuing a result, thereby ensuring the necessary provision to meet the clinical requirements for which the analyzer is intended. Its objective is to validate calibration and/or measurement processes and thus detect random errors that may affect results during the measurement process and obtain periodic statistical calculations of analytical imprecision²¹ for evaluation. For this purpose, a validated material is used, and if possible, with a matrix most similar to the biological system containing the constituents to be measured, which is usually supplied by the same commercial company as the analyzer.

Some analyzers use electronic quality control that, upon analyzer power-on or on demand, checks all electronic actions involved in the measurement process. This procedure is complementary and cannot replace classic quality control, which uses biological material simulating the sample.

In the case of POCT tests, there is no consensus on the frequency with which internal quality control should be analyzed. This frequency will depend on the importance of detecting measurement errors and the repercussions these may entail. Recommendations exist to do it from each work session (8 h)²² to weekly²³ or monthly, but also with each change of reagent lot and each opening of a new reagent box. Manufacturers of thromboelastography equipment recommend daily use of electronic controls and performing conventional quality control with validated material monthly for equipment with little use, or more frequently if the equipment is used more often.

For ROTEM and ClotPro® models, internal quality control²³ is recommended weekly, and for Sonoclot®,

a daily viscosity test and classic plasma quality control monthly are recommended. These are minimum recommendations, and it is advised that each center's multidisciplinary working group in charge of VETs establishes a frequency protocol for quality control analysis that ensures the perfect functioning of the analyzers based on their volume and frequency of work.

External quality control is known as an intercomparison program and involves comparing the results obtained by our center with the rest of the centers participating in the program to detect total error and bias of analytical procedures.²⁴

In this type of program, the control sample must be blind, i.e., of unknown value to the center at the time of analysis, and it may be appropriate to use a set of control samples with different values.

Currently, there are several options for external quality assessment programs for VETs. In the United Kingdom, the National External Quality Assurance Scheme (NEQAS, <https://ukneqas.org.uk>) has an intercomparison program for TEG® 5000 and ROTEM® Delta analyzers. This program uses lyophilized plasma samples.

Recently, this program has included cartridge-based analyzers, such as the TEG® 6S and ROTEM® Sigma. Other external quality assessment programs for these types of tests are the External Quality Control of Diagnostic Assays and Tests (ECAT) (<https://www.ecat.nl>) in the Netherlands and WEQAS (<https://www.weqas.com/services/poct-eqa/viscoelastic-haemostasis/>), all using lyophilized plasma as a sample.

As with any measurement of biological variables, both the management of internal quality control and participation in external quality programs are fundamental to demonstrating the validity and applicability/reliability of the results, and this applies to results obtained with VETs. It is recommended that all quality control records and records of actions taken with their results be kept for a minimum period of two years for consultation and possible audits.

QUALITY CONTROL RECOMMENDATIONS

- Perform internal quality controls at least with the frequency recommended by the manufacturer. At least one weekly control is advised.
- It would be advisable to participate in an external quality program that allows for intercomparison and accuracy of results.

- It is advised that the laboratory, as an expert in the management and evaluation of analytical control programs, be responsible for the management of quality controls, through the POCT coordinator, if a hospital POCT committee exists, or by laboratory personnel designated for this purpose. For traceability and record-keeping, as indicated by the POCT system implementation guidelines,²⁵ it is advisable to establish an IT connection with POCT management systems and/or the laboratory's IT system for patient registration and quality control, and through them with the IT system that manages the hospital's health records for viewing and consulting results.

Precision and accuracy of results

Results obtained in external quality assessment programs (NEQAS)²⁶ show very large coefficients of variation (CV), both for pipetting-based instruments, such as the ROTEM® Delta (CV range depending on parameter 7.2%-73.2%) and TEG® 5000 (CV range depending on parameter 17.4%-59.5%), and for cartridge-based instruments, such as the ROTEM® Sigma (CV range depending on parameter 3.9%-22.9%) and TEG® 6S (CV range depending on parameter 13.6%-30.7%). This wide variation may be for 2 reasons. The first is a pre-analytical factor related to the variability introduced by user manipulation. Vials are supplied lyophilized and must be reconstituted by the user. The skill and accuracy of pipetting can affect the final result.²⁷

This argument is reinforced by observing that instruments with cartridge technology, where there is no pipetting by the operator, have lower CV than those that do. But even so, sample handling remains important for all devices. This type of variability can also be transferred to the analysis of patient samples, highlighting the importance of the pre-analytical phase in performing these types of tests, emphasizing the crucial role of training and acquiring expertise with the materials used by the personnel who will perform the measurements on patients.

The second reason may lie in the use of lyophilized plasma samples in these intercomparison programs, instead of whole blood, as the devices are not designed to evaluate plasma. Currently, no provider of external quality programs can provide "fresh whole blood" or similar material as samples within their programs. In summary, lyophilized samples can be used to demonstrate test precision and allow peer comparison between different centers but cannot be used to guarantee accuracy.²⁸

Reference intervals

VETs are poorly standardized. Reports demonstrate the non-interchangeability of results obtained with thromboelastographic techniques and those obtained with thromboelastometry.²⁹

In addition to the reference intervals provided by manufacturers, no consensual reference values have been published. As with any laboratory test, centers should determine their own reference values, based on the characteristics of their local population. This is particularly difficult in the paediatric population. Some authors have established reference values for this type of patient for ROTEM® Delta⁴ and for TEG® 5000.³⁰

Implementation model of VETs

Obtaining rapid results for urgent clinical decision-making that allow early recognition of critical situations, faster therapeutic intervention, or reduction of perior postoperative complications are some important objectives of POCT.

VETs, due to their nature and immediate clinical application, are tests susceptible to being performed at the patient's bedside; however, they have always had certain limitations that have conditioned their use to relatively reduced clinical environments.

Pipetting of the sample and reagents, the relatively complex interpretation of results, and even the size and weight of the equipment, have posed significant barriers when implementing this technology at the patient's bedside in certain clinical units.

In search for the clinical utility of this methodology, diagnostic companies have implemented important developments in their devices that have allowed them to overcome the main obstacles and bring this technology to increasingly complex clinical environments, adapting to the clinical-care and organizational needs of different centers.

The use of VET at the point of patient care (POCT) provides speed and immediacy in performing the test and visualizing the results at the same location as the patient, thus reducing response and action times in a critical bleeding situation. However, it also exposes the procedure to a multitude of factors that can adversely affect sample analysis: the handling of analyzers by multiple, potentially unqualified operators, limited pre-analytical knowledge, and inadequate quality control management.²⁵

On the other hand, there is the centralized model, where VETs are performed in the emergency

laboratory, which has been implemented in many hospitals. This model offers a series of advantages: service available to the entire hospital, managed quality control, avoidance of interference from the movement of vibration-sensitive equipment (moving analyzers with carts from one place to another), and specialization of personnel performing the technique and inclusion in the patient's clinical history (this can also be done in POCT). The visualization of results is done remotely and in real time electronically and can be viewed simultaneously across different workstations or locations. This model involves perfect coordination between services, establishing consensual work protocols that allow reducing the time to obtain results, which is its main disadvantage.

In other hospitals, a mixed model has been chosen, with some analyzers in high-activity units (POCT) and other analyzers in the emergency laboratory, serving as support and service to the entire hospital. Therefore, each hospital center must implement the model that best suits its needs, advising that this implementation always be carried out with the control and management of a hospital POCT committee.

Description and current scientific evidence on the use of platelet function tests. Description of existing tests. Mapping using ROTEM®, TEG®, and Quantra®

In the context of coagulopathy, evaluating the contribution of platelets to hemostasis is fundamental. The role of platelets in primary hemostasis for clot generation, providing a surface for prothrombin-to-thrombin conversion, and catalyzing processes in secondary hemostasis, stabilizing the clot through fibrin mesh formation and improving clot firmness through clot contraction, thereby also restoring blood circulation, is well-known. There are various methods for evaluating platelet actions such as adhesion, aggregation, secretion, clot retraction, platelet morphology evaluation, etc. In clinical practice, platelet counts are frequently performed, but the evaluation of their functionality is less common. The difficulty of functional evaluation lies in the tests' ability to recreate the complex interactions that occur *in vivo* with the interaction of various mediators and the dynamic flow and shear conditions to which platelets are subjected. It should be noted that it is assumed before the test that platelets are inactive and will be activated after agonist exposure; however, at the time of the test, they may have already been

activated and apparently show impaired function in the *ex vivo* test.³¹ Bedside devices for diagnosing hemostatic status allow for targeted and early treatment, as well as avoiding unnecessary transfusions, by reducing response time compared to central laboratory analyses. Platelet count is used in the decision for platelet transfusion in this context; however, it has the limitation of delayed results from the central laboratory and does not detect functional defects that may be present despite normal counts. Various systems allow their use at the patient's bedside for functional assessment of platelet function in addition to counting, thus reducing diagnosis and treatment time.

Various systems are used for the evaluation of platelet function. Some are based on platelet aggregation, such as light transmission aggregometry (LTA), VerifyNow, or Plateletworks; others are based on platelet adhesion due to shear stress, such as PFA or the Impact-R system; others on flow cytometry analysis; others on the evaluation of thromboxane metabolites; and finally, those based on combined methods with global clot formation VETs. The main characteristics are shown in [table 6](#).

Not all can be used in the bleeding setting and they also vary depending on the need for processing. They are influenced by platelet count, although there is the possibility of analyzing platelet function with methods adjusted to the existing count.³² Overall, they are based on exposing the platelet to an agonist such as collagen, arachidonic acid (AA), epinephrine, adenosine diphosphate (ADP), thrombin, or thrombin receptor activating peptide or other activators of different pathways. That is, a limited number of platelet activation pathways can be explored simultaneously.

As a general rule, whenever a platelet function test is to be performed, pre-analytical platelet activation must be avoided, and for this, certain precautions are necessary in relation to the patient and the extraction technique. There are patient-related factors such as prior exercise, certain foods and supplements, coffee consumption, drugs such as non-steroidal anti-inflammatory drugs, or circadian rhythm. The extraction should be performed gently, with the minimum necessary tourniquet pressure and needles > 19 G with short connection tubes. Additionally, it is recommended to discard the first milliliters of the extraction to avoid activating substances due to endothelial injury. The type of anticoagulant used is also important, with citrate showing lower values of platelet aggregation,³³ and for transport, it is recommended to avoid pneumatic systems and to transport tubes in an upright position, avoiding agitation.

Table 6. Main characteristics of systems for platelet function evaluation

Test	Method	Advantages	Disadvantages	Notes
LTA	Aggregation measured by photo-optical transmission. Citrated sample or PRP	Identifies specific deficiencies, whether genetic or due to antiplatelet agents	Technically complex; requires expert interpretation. Not a POCT	Reference method. Uses different agonists activating various pathways
Multiplate analyzer®	Aggregation based on impedance	Can be used as POCT. Uses whole blood. Automated. Can detect antiplatelet effect	Inconsistent correlation with LTA. Uses anticoagulated blood under low shear conditions, unlike physiological conditions. Highly dependent on platelet count	Low platelet reactivity: < 19-30 U
Plateletworks®	Aggregation (difference in count before and after activation with agonists). Citrated whole blood	Small sample. Detects antiplatelet effect. Useful for monitoring	Requires calibration and manual processing. Only for antiplatelet monitoring. Interference in pseudothrombocytopenia, large platelets, and cell fragments	Scale ranges from 100% aggregation to 0% inhibition. Low platelet reactivity: < 80%
PFA®-100/200	Evaluates adhesion and aggregation (closure time, high shear condition). Citrated whole blood	POCT potential. Detects platelet dysfunction and von Willebrand disease. Sensitive to antiplatelet agents. Assesses bleeding risk	Results affected if platelet count < 50,000 or hematocrit < 25%	
VerifyNow®	Aggregation by optical turbidimetry	POCT, uses whole blood without processing. Detects antiplatelet effect. Automated with specific cartridges (AA, ADP/PG E1, TRAP)	Only evaluates effects of antiplatelet drugs. Sensitive to thrombocytopenia, lipidemia, anemia, and fibrinogen levels	Low platelet reactivity: < 85 PRU
TEG® (Platelet Mapping® module)	Global coagulation test. Evaluates activation and aggregation by MA and MA AA/ADP difference. Citrated whole blood	POCT with rapid results. Detects antiplatelet drugs	Identifies specific platelet function disorders	Low platelet reactivity defined as < 31 mm TEG MAADP or > 50–60% drop in MAADP vs TEG-MA kaolin
ROTEM® (ROTEM Platelet module)	Global coagulation test. Evaluates activation and aggregation via impedance aggregometry. Citrated whole blood	POCT with rapid results. Detects antiplatelet effect with 3 tests: ADPTM, ARATEM, TRAPTEM	Does not identify specific platelet function disorders	Limited device availability
Quantha®	Sonic estimation of elasticity by resonance (SEER sonorheometry)	Directly reports platelet contribution using PCS value, which is given in shear modulus units	Correlations and cut-offs in patients on antiplatelet agents are yet to be defined	Treatment threshold PCS < 75%

LTA: light transmission aggregometry; PCS: platelet contribution to clot stiffness; POCT: Point-of-Care test.

The analysis should ideally be performed between 30 minutes after collection and before 3-4 hours have passed, and it should be kept at room temperature.

Use of VETs

VETs use whole blood samples and provide information on the kinetics of clot formation from its initiation,

the generation of the platelet clot, its stabilization, and lysis. Therefore, unlike aggregation and cytometry methods that evaluate platelet function indirectly or via surface proteins, VETs assess the contribution of platelets and coagulation factors to this global clot formation by evaluating clot firmness or amplitude. In VETs, clot firmness is measured after platelet activation by thrombin and its polymerization with fibrin; thus, the

impact of platelet count on elasticity is very high.¹² These tests incorporate methods for inhibiting platelet function to eliminate the contribution of platelets to clot formation, thereby aiming to interpret their degree of function. In general, platelet contribution represents 80% of the clot firmness obtained in these tests.³⁴

Another relevant aspect is the relationship of platelet adhesion to hematocrit, making it a factor to consider in the results when evaluating platelet functionality.³⁵

The differing terminology among devices also causes confusion. For example, the maximum clot firmness (stiffness), which serves as a reference, is expressed in various ways: as MCF in ROTEM, MA in TEG, and CS (clot stiffness) in Quantra®. The latter also indicates values in terms of shear modulus (hPa), unlike the first two. From these MCF, MA, or CS values, platelet contribution could be established by eliminating fibrinogen's contribution to clot formation. However, using the difference between total clot amplitude and clot amplitude with only fibrinogen contribution would introduce a bias that underestimates the true platelet contribution. To avoid this bias, platelet contribution should be estimated by comparing amplitudes using shear modulus differences. The shear modulus (or elasticity) is not linearly associated with clot amplitude and would be better defined by this equation:

$$\text{Shear modulus (G, dyn/cm}^2\text{)} = (5,000 \times \text{amplitude [mm]}) / (100 - \text{amplitude [mm]})$$

This formula represents an exponential curve in the relationship, meaning that the greater the clot amplitude, the greater the difference between both measurements. For this reason, in bleeding patients with low amplitudes, platelet contribution has significant clinical relevance.³⁶

Thus, for ROTEM and TEG, this estimation of platelet contribution cannot be performed directly from the obtained stiffness values; it requires conversion to G, whereas Quantra® already expresses it in this unit.

It has also been shown that a linear relationship exists between changes in platelet counts and the maximum firmness results of VETs for platelet ranges between 28,000 and 91,000; however, outside this range, the changes are not dependent.

Regarding platelet inhibition, an association is only found with inhibition degrees between 68% and 82%, with VETs being insensitive to different inhibition degrees. Therefore, the viscoelastic method is considered to precisely evaluate antiplatelet-mediated inhibition and requires the development of tests with specific reagents. Another limitation is its performance under low shear conditions, which prevents the

identification of specific platelet dysfunctions. Thus, manufacturers of POCT VETs have developed different strategies to identify platelet functionality.

TEG® PLATELET MAPPING® ASSAY (TEG® 6S)

The TEG system incorporates a module, the PlateletMapping®, which automatically monitors platelet aggregation specifically alongside the VET. After heparinizing the sample to prevent clot formation, it allows antiplatelet monitoring through selective activation of the P2Y₁₂ receptor with ADP or the thromboxane pathway with AA. This enables measurement of the response to antiplatelet agents acting on these pathways. In the case of glycoprotein IIb/IIIa inhibitors, both the ADP test and the AA test are affected. This method has been validated against light transmission aggregometry as a reference method. First, maximum hemostatic activity is determined with a kaolin test, and then reptilase, factor XIIIa, and AA or ADP are added to a whole blood sample. Inhibition of platelet activation is calculated by comparing the kaolin test result as a reference; that is, the platelet contribution to global clot formation is obtained by comparing results with those obtained with TEG, but primarily aimed at identifying effects of inhibition by antiplatelet agents.

In settings such as trauma patients or cardiovascular surgery, it allows identification of patients with the effect of previous antiplatelet agents, although it has not been shown to have predictive value on mortality by itself.³⁷ It has shown predictive capacity for thrombotic and hemorrhagic risk in both cardiac and non-cardiac surgery patients.^{38,39}

In patients on dual antiplatelet therapy preoperatively, platelet function testing has been used to determine the surgical scheduling date. Thus, using MA_{ADP} reduces surgical delay time by 50% vs traditional suspension regimens.⁴⁰ Other described uses in cardiovascular surgery patients include association with VETs to improve the predictive capacity for perioperative bleeding, prediction of transfusion requirements, and guidance for antiplatelet therapy in patients with left ventricular assist devices.⁴¹

Overall, a cutoff point for proceeding or not with surgical intervention in antiplatelet patients is considered to be a 35% inhibition of ADP-induced aggregation.³⁹ Low platelet reactivity is defined by values < 31 mm TEG MA_{ADP} and in special circumstances, such as patients with circulatory assistance, values < 40 mm TEG MA_(activator).⁴² Various studies have suggested the utility of using TEG PlateletMapping® preoperatively

in antiplatelet patients to determine the moment of intervention and reduce hospital stay and costs.⁴³

As with other platelet function tests, other uses have also been described, such as monitoring the response to antiplatelet agents to individualize the type and dose of antiplatelet agents in patients who require it due to cardiac and cerebral vascular disease.

ROTEM® PLATELET ASSAYS

The platelet module is an add-on to the ROTEM machine that facilitates the analysis of platelet aggregation using an impedance aggregometry method.⁴⁴ It complements ROTEM's clot formation assessment, which does not detect the presence of platelet inhibitors.⁴⁵ It uses agonists ADP and AA in the tests respectively named ADPTEM and ARATEM; furthermore, it has a test that uses thrombin receptor activating peptide called TRAPTEM.

Studies have shown that the sample should rest for 15 minutes before analysis, and when a citrated sample is used, it should be evaluated within the following 60 to 120 minutes.⁴⁶ Reference intervals have been described for the impedance aggregometry method using other devices⁴⁷, and more recently, studies have been conducted to validate these intervals on the ROTEM® Platelet equipment.^{46,48}

Of note, the common use of citrated samples for study can cause artifacts in the *in vitro* detection of secondary platelet aggregation due to calcium depletion.⁴⁹ The use of tubes with hirudin instead of citrate can overcome this drawback. Nevertheless, some studies have established reference intervals for ADPTEM, ARATEM, and TRAPTEM, considering whether citrated or hirudin tubes are used.⁴⁶

Clinically, it has demonstrated utility in evaluating bleeding and transfusion risk in certain surgical settings.^{50,51}

QUANTRA® SYSTEM

This system is based on the sonorheometry method, using an ultrasound system to estimate elasticity by resonance (SEER) and thus evaluate the viscoelastic properties of whole blood. This system can provide more specific information than other VETs regarding platelet contribution to clot strength.⁵² However, studies establishing correlation with platelet function in patients with P2Y12 receptor inhibitors and determining cutoff points are necessary, as the PCS parameter actually represents a combination of platelet count and function.

The advantage of Quantra® PCS is that it directly shows platelet contribution via shear modulus difference, without needing to convert obtained values to shear modulus to infer the true platelet contribution, as occurs with other VETs. In general, a PCS value < 75% is considered a cutoff point for treatment, taking into account the clinical situation.

The PCS directly informs about platelet contribution to clot formation during the study. An independent association has been demonstrated between the values of PCS and platelet count and ADP-mediated platelet function.⁵² In clinical studies, this parameter has shown predictive capacity for the need for platelet transfusion in major surgery.⁵³

Conclusion

Evaluating platelet functionality represents a challenge due to the difficulty in recreating physiological *in vitro* conditions and the complex interaction of platelets with different receptors and mediators. Furthermore, there are significant differences in comparisons between various tests due to differences in methods and agonists used. All of this means that standardization guidelines have not been established, and there is significant variability in use and indications.

Economic impact of VETs use: savings in blood components and length of stay

The use of VETs should not be seen as an isolated intervention in the management of bleeding patients; rather, it should be framed within a much broader and more current context: patient blood management (PBM).

The World Health Organization (WHO) published a concise yet forceful document in 2021 on the urgency of implementing PBM programs after experiencing situations of tremendous scarcity of blood resources during the COVID-19 pandemic, which led to drastic reductions in donation volumes.

PBM is often identified with the correction of anemia and optimization of hemoglobin levels through iron and hematinic administration (PBM pillar 1). However, anemia can also be associated with medical and surgical processes and is linked to increased morbidity and mortality, prolonged length of hospital or ICU stays, and reduced quality of life.⁵⁴

VETs play a role precisely in those bleeding situations associated with surgical trauma, childbirth, or coagulopathies; they enable addressing the second

pillar of PBM, focused on minimizing blood loss and correcting coagulation. The results of the blood management program in Western Australia between 2008 and 2014 showed a 15% reduction in hospital stay and an economic saving of 18.5 million dollars in blood products.

Managing bleeding and efficiently and promptly correcting coagulation disorders are important, first and foremost for patient safety reasons, but also concerning the saving of health care costs and the sustainability of health care systems.

It is relevant to note the official prices (average prices) of blood components, VETs, and standard laboratory tests to determine the scope of savings with the use of VETs.

The rates published in the official gazettes of different autonomous communities are quite similar for the final price of blood components ready for transfusion, being €600 per platelet concentrate, €300 per red blood cell concentrate, and approximately €60 per unit of fresh plasma. Performing a global hemostasis VET with a cartridge system (ROTEM® Sigma, TEG6®, Quantra®) has an average cost of €100, while if ROTEM® Delta is used, each isolated determination costs about €25. Determining platelet function with cartridges on TEG® 6S costs €120. On the ROTEM® Deltadevice, each platelet receptor studied (AA, ADP, thrombin) costs €16.

Standard coagulation tests are cheaper than VETs (although the price varies in each hospital), but they do not provide immediate or rapid results and delay decision-making.

Transfusion of blood components is, *per se*, an incremental factor of morbidity, mortality, and length of stay,⁵⁵ with a proportional relationship between transfused volume and complications.⁵⁶ The saving of blood components already justifies the use of VETs *per se*, but there is also a reduction in care costs by reducing hospital stay. Unfortunately, it is difficult to establish exact calculations of these savings, although it can serve as a guide that the daily net cost of a patient in a critical care bed in our country varies between €800 and €1,500 (depending on the autonomous community), without accounting for personnel costs or administered drug.

The best-studied clinical scenario in the field of VETs is cardiac surgery, and numerous publications have demonstrated a reduction in transfusion volume and reoperation rates due to bleeding. The issue of savings in coagulopathy management in cardiac surgery was already addressed years ago by Spalding⁵⁷ (in 2007), with reductions of 25% in red blood cell consumption,

50% in platelet consumption, 80% in prothrombin complex concentrate and factor XIII, with cessation of recombinant activated factor VII use and, in contrast, doubling fibrinogen use. Spalding calculated a monthly cost reduction in blood products of €21,000, with an average cost of ROTEM® reagents and consumables of €1,580, and a reduction in the rate of re-sternotomies due to bleeding from 6.6% to 5.5%.

Of note, these benefits are enhanced if the use of VETs is associated with a transfusion strategy guided by algorithms. Karkouti et al.,⁵⁸ in 2016, demonstrated very significant reductions in bleeding rates, red blood cell and platelet transfusions, and a 26% decrease in reoperations at a time that coincided with increased complexity of the surgical cases.

The IMOTEC study,⁵⁹ whose results are pending publication, aims to evaluate the cost-effectiveness of using an algorithm-based VET and its impact on quality of life one year after surgical intervention, with special emphasis on the calculation of hospital costs per patient.

Whiting et al.⁶⁰ in their cost-effectiveness analysis of VET use in various scenarios, concluded that in cardiac surgery, the consumption of red blood cells, platelets, and plasma was reduced with the use of these tests, and they quantified a saving per patient of £43 with ROTEM®, £79 with TEG®, and £132 with Sonoclot®. In polytrauma patients, the calculated savings were greater: £688 with ROTEM®, £721 with TEG®, and £818 with Sonoclot®.

González et al.⁶¹ published interesting reductions in 28-day mortality with the use of VETs to guide transfusion therapy in polytrauma patients vs standard laboratory guidance.

A systematic review and meta-analysis by Soares⁶² in 2020 directly linked the use of VETs with a significant reduction in mortality, development of acute kidney injury, volume of administered red blood cells, and risk of platelet and plasma transfusion in surgical scenarios in general.

It is not easy, but it is common sense, to extrapolate that there is considerable savings if we avoid a patient entering renal replacement therapy through appropriate use of hemostatic and transfusional resources.

A 2014 review⁶³ extensively described many economic aspects of healthcare, which involves direct costs derived or not derived from healthcare (e.g., blood products and drugs are direct costs derived from healthcare, while transportation, social services, or legal fees are not derived from health care but do incur direct costs) and also indirect costs (productive losses due to

absenteeism or disability or morbidity and mortality). Clearly, the calculation is much more complex than it might seem *a priori*.

Clinical scenarios such as liver transplantation show contradictory evidence in the scientific literature regarding the usefulness of VETs, although their use is widespread, and they constitute a fundamental theragnostic tool in complex cases tending towards both hemorrhage and thrombosis. Randomized clinical trials by Bonnet⁶⁴ and Zamper⁶⁵ demonstrate a reduction in red blood cell and plasma transfusion with the use of thromboelastometry, although neither reaches conclusions of advantage regarding mortality, reoperation rates, or hospital stay.

Finally, in postpartum hemorrhage,⁶⁶ a reduction in plasma consumption and a slight tendency towards less bleeding have been demonstrated with the use of ROTEM to guide hemostasis.

Many other situations in clinical practice can benefit from the use of VETs and platelet function mapping, such as interventional radiology procedures involving vascular stent placement or large burn units, which undergo numerous fasciotomy and debridement interventions with a significant tendency to bleed. The evidence in these cases is very scarce.

In conclusion, the use of VETs is cost-efficient not only from the perspective of economic savings in blood components, which are expensive and scarce, but especially from the perspective of patient safety and optimizing their clinical outcome. The strict calculation of expense is, however, complex and multifactorial.

Funding

None declared.

Conflicts of interest

None declared.

Ethical considerations

Protection of persons and animals. The authors declare that no experiments on human beings or animals were conducted for this investigation.

Confidentiality, informed consent, and ethical approval. The study does not involve personal patient data and does not require ethical approval. SAGER guidelines do not apply.

Statement on the use of artificial intelligence. The authors declare that they did not use any type of generative artificial intelligence for the writing of this manuscript.

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Factor VIII, IX, and XI measurement by one-stage clotting assay with silica clotting time confirm reagent: a clinical laboratory helpful tool in the presence of lupus anticoagulant

Dosaje de factores VIII, IX y XI en un ensayo coagulable en una etapa utilizando reactivo de tiempo de sílica confirmatorio: una herramienta clínicamente útil ante la presencia de anticoagulante lúpico

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Abstract

Introduction: In clinical practice, coagulation factors levels measurement is used to detect possible factor deficiency in patients with bleeding manifestations or to explain the origin of prolonged routine coagulation tests results. **Objectives:** The aim was to evaluate factor VIII (FVIII), factor IX (FIX), and Factor XI (FXI) assay performed with silica clotting time confirm (SCTC) reagent in LA positive (LA+), normal controls (NC), and factors deficient (D) samples. **Materials and methods:** Citrated plasmas: 73 LA+, 45 NC; 77 D: 40 FVIII, 22 FIX, and 15 FXI. FVIII, FIX, and FXI D. (1) SynthASil activated partial thromboplastin time (APTT) (SYNT); (2) SCTC (3) FVIII chromogenic assay (FVIIICHROM). **Results:** NC: slightly lower FVIII SCTL than FVIII SYNT and FIX SYNT than FIX SCTL. No differences FIX SYNT versus FIX SCTL. D: No differences between FVIII SCTL, FVIII SYNT, and FVIII CHROM, or FIX SYNT versus FIX SCTL. FIX SYNT is slightly higher than FIX SCTL. NC+C: FVIII, FIX and FXI SCTL-SYNT $r > 0.970$. LA+: 36.7% FVIII SYNT, 66.1% FIX SYNT, and 86.2% FXI SYNT nonparallel dilution curves. FVIII SYNT1/40, FVIII SCTL, and FVIII: CHROM comparable results. FVIII SCTL-FVIII CHROM, $r: 0.949$, FVIII SCTL-FVIII SYNT, $r: 0.759$. Low FIX SCTL-FIX SYNT, no correlation FIX SCTL-FIX SYNT. **Conclusion:** FVIII, FIX, and FXI assays with SCTC seem to be useful to measure factor levels in LA+ samples.

Keywords: Coagulation factors dosage. Lupus anticoagulant. Silica clotting time confirm.

Resumen

Introducción: En la práctica clínica, la medición de los niveles de los factores de coagulación se utiliza para detectar posibles deficiencias de factores en pacientes con manifestaciones hemorrágicas o para explicar el origen de resultados prolongados en las pruebas rutinarias de coagulación. **Objetivo:** Evaluar dosajes de FVIII, FIX y FXI con Sílica Clotting Time Confirm (SCTL) en muestras anticoagulante lúpico-positivas (LA+), controles normales (CN) y deficientes de factores (D).

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Date of reception: 14-02-2025

Date of acceptance: 17-05-2025

DOI: 10.24875/RHT.M25000065

Available online: 18-09-2025

Hemost Trombos (Eng). 2025;2(2):60-67

www.revistahemostasiaytrombosis.com

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Materiales y métodos: *Plasmas citratados: 73 LA+, 45 CN y 77 D: 40 FVIII, 22 FIX y 15 FXI. Dosaje FVIII, FIX y FXI: (1) APTT-SynthASil (SYNT); (2) SCTC (3) FVIII Cromogénico (FVIII CHROM).* **Resultados:** *CN: FVIII SCTC ligeramente inferiores a FVIII SYNT y FIX SYNT a FIX SCTC. FIX SYNT-FIX SCTC comparables. D: Sin diferencias FVIII SCTC, FVIII SYNT y FVIII CHROM, ni FIX SYNT versus FIX SCTC. FIX SYNT ligeramente superior a FIX SCTC. CN + D: $r > 0.970$ FVIII, FIX y FXI SCTC-SYNT. LA +: curva de dilución no-paralela 36.7% FVIII SYNT, 66.1% FIX SYNT y 86.2% FXI SYNT. FVIII SYNT1/40, FVIII SCTC y FVIII CHROM comparables. FVIII SCTC-FVIII CHROM $r = 0.949$, FVIII SCTC-FVIII SYNT $r = 0.759$. Baja FIX SCTC-FIX SYNT y no correlación FIX SCTC-FIX SYNT.* **Conclusión:** *Los ensayos de FVIII, IX and XI con SCTC parecen útiles en muestras LA+, especialmente cuando se necesitan altas diluciones.*

Palabras clave: *Dosaje de factores de coagulación. Anticoagulante lúpico. Tiempo de Sílica confirmatorio.*

Introduction

In clinical practice, coagulation factors levels measurement is used to detect possible factors deficiency in patients with bleeding manifestations, or to explain the origin of prolonged routine coagulation tests results. Particularly important are the intrinsic pathway factors assays, due to the highly prevalent severe bleeding disorders like hemophilia A, factor VIII (FVIII) deficiency, and B, factor IX (FIX) deficiency not only for the diagnosis but also for replacement therapy monitoring. FVIII is also important for von Willebrand disease diagnosis, and Factor XI (FXI) for diagnosis and therapy monitoring of FXI deficiency¹. The most widely available assay is the clotting one-stage method based on activated partial thromboplastin time (APTT) assay performed on a patient plasma dilution (PD) and factor-specific deficient plasma mixture¹⁻³. It is recognized that different APTT reagents (phospholipid [PL] origin, composition, and concentration, and intrinsic pathway activator) show different sensitivity to specific factor levels as well as to the presence of lupus anticoagulant (LA) activity in plasma. The presence of LA leads to false low levels factors due to the interference of LA on the PL availability for the APTT test used in the assay. It is suggested that APTT reagent used for factor assays should contain a high PL concentration and be low sensitivity to LA⁴. It has also been recommended that factor assays must be performed by processing in at least three patient PD and analyzing results by the parallel-line method in order to obtain a more accurate result and also to detect and overcome interferences like LA^{1,5}. Other techniques have been suggested to eliminate LA interference in factors activity measurements like neutralization of LA interference by adding hexagonal phase PL to patient plasma previous factor testing⁶, use of heterophilic factor deficient plasmas instead of human deficient ones⁷, or using amidolytic or chromogenic (CHROM) assay^{1,8}. Of these, CHROM methods are available in many hemophilia centers but not in clinical laboratories.

Depending on the characteristics and concentration of antibodies, many LAs generate a very long prolongation in APTT and could interfere with factor dosing even at 1:40 PD (the highest dilution performed automatically by some coagulometers), causing falsely low results that force the process several additional PD to reach the actual factor level with less accurate results.

Some authors have been reported that using confirmatory (high PL) silica clotting time confirm (SCTC) reagent in assays for intrinsic pathways factors could be a good and easy tool to overcome LA effect⁹.

The present study aimed to evaluate FVIII, FIX, and FXI assay performed with an APTT, which contains silica as activator and high PLs concentration (SCTC) in a large number of LA positive samples as well as in normal controls (NC) and factors deficient plasmas in three different laboratories to verify the previously reported study.

Materials and methods

Population

A total of 195 samples were evaluated. Samples and controls evaluated are listed in [table 1](#). The study was performed by three different hemostasis laboratories from different hospitals: 1-Hospital Italiano de Buenos Aires, 2- Unidad Asistencial Por más salud Dr, César Milstein, 3-Hospital Británico de Buenos Aires, all in Ciudad Autónoma de Buenos Aires, Argentina. LA positive samples were mainly from center 1, samples from congenital deficient patients were collected in all three centers, but hemophilia A (HA) samples were mainly from center 2. The protocol was revised and approved by the Institutional Review Board on human subject research of the Hospital Italiano de Buenos Aires Ethics Committee (CEPI). No written consent was required because the study fulfilled the established criteria for handling shed blood samples and, patients' data were collected retrospectively, identifiers were removed, and data used was anonymous.

Table 1. Study and control groups description

LA+		NC	FVIIIID		FIXD		FXID	
Description	n	n	Description	n	Description	n	Description	n
Total	73	45	Total	40	Total	22	Total Congenial deficient total	15
LA+	60		HA	30	Mild HB	6	Severe	8
LA+AVK	13		Severe HA	11	Vitamin K deficient	2	Moderate	1
			Moderate HA	9	AVK	14	Mild	6
			Mild HA	10				
			VWD	6				
			Acquired HA	4				

LA+: lupus anticoagulant; NC: normal controls; FVIIIID: factor VIII deficiencies; FIXD: factor IX deficiencies; FXID: factor XI deficiencies; AVK: vitamin K antagonists; HA: hemophilia A; HB: hemophilia B; VWD: von Willebrand disease.

Blood sampling

Blood was drawn by clean venipuncture and collected in vacutainer tubes containing 1/10 volume of sodium citrated (Becton Dickinson). Platelet-poor plasma was obtained by centrifugation at room temperature of 200 g for 10 min. A second plasma centrifugation was performed, and samples were divided into 2 aliquots: One was processed as per the physician's request, and the other was kept frozen at -70°C until used for the study (no more than 3 months).

Coagulation tests

For APTT assay: HemosIL APTT SP (Werfen).

The two methods used for factor dosages were performed using FVIII, IX, and XI immune deficient plasmas and HemosIL Calibrator Plasma, both from Werfen.

- Standard method: FVIII SYNT, FIX SYNT, and FXI SYNT dosage in 3 PDs (1/10, 1/20, and 1/40), using HemosIL SynthASIL APTT reagent (Werfen). All laboratories used two levels of internal controls (normal and low) (HemosIL NC assayed and HemosIL special test 2 control, Werfen and participated in an external proficiency program).
- Alternative methods FVIII SCTC, FIX SCTC, FXI SCTC: 1/10 PD, HemosIL SCT confirm (SCTC) as APTT reagent, and HemosIL SCT CaCl_2 both from Werfen. The method was validated using two levels of internal controls and compared with the previous method.
- FVIII CHROM assay (FVIII: CHROM): Electrachrome FVIII Kit (Werfen) containing bovine FIXa 0,3 U/vial, FX in excess 2,7 IU/vial and Thrombin 1 UNIH/vial, CaCl_2 40 μmol /vial and PLs 0.2 μmol /vial). CHROM

substrate: S-2765 7,7 mg/vial with thrombin synthetic inhibitor I2581 and mannitol reconstituted in tampo-nade 25mM TRIS.

All coagulation tests were performed in ACL TOP family coagulometers: ACLTOP 500 (Werfen).

Statistics

Friedman and Wilcoxon pared non-parametric tests. Correlations were performed by the Spearman Rank method. Statistics performed by IBM Statistical Package for the Social Sciences 23 software.

Results

Analytical inter-assay coefficient of variation for SCTC methods were: FVIII 4.5% and 6.3%, FIX 4.9% and 7.0%, FXI 4.7% and 6.1% comparable to that observed by the original assay used in (SYNT): FVIII 4.5% and 3.5%, FIX 5.2 and 4.7%, FXI 5.3 and 8% for normal and low abnormal controls, respectively.

Comparison of FVIII, FIX, and FXI levels measured between SYNT and SCTC methods in NC and factor deficiencies

Table 2 shows the results of FVIII, FIX, and FXI obtained by different methods NC, as well as FVIIIID, FIXD, and FXID (control samples). In NC, FVIII SCTC showed statistically slightly lower values than FVIII SYNT without clinical significance. In the case of samples from FVIIIID, no statistical differences were observed between FVIII SYNT and FVIII SCTC (Fig. 1). All NC

Table 2. FVIII, FIX and FXI levels measured in LA + samples and control groups

Sample type	FVIII SYNT (IU/dL) 1/10 Median (IQR)	FVIII SYNT (IU/dL) 1/40 Median (IQR)	FVIII SCTC (IU/dL) 1/10 Median (IQR)	FIX SYNT (IU/dL) 1/10 Median (IQR)	FIX SYNT (IU/dL) 1/40 Median (IQR)	FIX SCTC (IU/dL) 1/10 Median (IQR)	FIX SYNT (IU/dL) 1/10 Median (IQR)	FIX SYNT (IU/dL) 1/40 Median (IQR)	FIX SCTC (IU/dL) 1/10 Median (IQR)	FXI SYNT (IU/dL) 1/10 Median (IQR)	FXI SYNT (IU/dL) 1/40 Median (IQR)	FXI SCTC (IU/dL) 1/10 Median (IQR)
Normal controls	130 (103-161)			106 (92-120)			106 (92-120)		113* (100-137)			105 (100-127)
LA +	97 (79-128)	109 [†] (84-140)	106 [§] (89-126)	59 (25-84)	83 (59-105)	102 [¶] (87-115)	46 (20-98)		83** (49-118)			105** (93-118)
FVIII	3.6 (0.9-32.6)		4.8 (1.1-28.9)									
FIXD				37 (24-44)		32 ^{††} (19-45)						
FXID										1.0 (1.0-15.0)		1.4 (0.0-11.5)

*p = 0.002 FVIII SYNT 1/10 versus FVIII SCTC 1/10.

†p = 0.041 FIX SYNT 1/10 versus FIX SCTC 1/10.

‡p < 0.001 FVIII SYNT 1/10 versus 1/40.

§p = 0.046 FVIII SYNT 1/10 versus FVIII SCTC 1/10; and p < 0.001 FIX SYNT 1/40 versus FIX SYNT 1/10 and FIX SCTC 1/10 versus FIX SYNT 1/10.

¶p = 0.001 FIX SCTC versus FIX SYNT 1/40.

**p < 0.001 FXI SYNT 1/10 versus FXI SYNT 1/40 and FIXSCTC 1/10 versus FXI SYNT 1/10.

††p = 0.011 FIX SCTC 1/10 versus FIX SYNT 1/10.

LA: lupus anticoagulant; FVIII: FVIII deficient; FIXD: FIX deficient; FXID: FXI deficient; IQR: interquartile range.

and D plasmas presented parallel dilution curves (DC) compared to normal plasma. FIX SCTC presented statistically slightly higher values than FIXSYNT in NC but statistically lower values in FIX deficient (Table 2 and Fig. 2A), without clinical significance in both cases. There were no differences between FIXSYNT and FXI SCTC in NC nor in FIXI deficient (Table 2, Fig. 2B). The correlation between SCTC and SYNT methods considering normal and deficient plasmas were 0.975, 0.977, 0.995 for FVIII, FIX and FXI, respectively, as well as between FVIII SCTC and FVIII: CHROM, 0.968, p < 0.001 for all correlations.

Comparison of FVIII, FIX, and FXI levels measured between SYNT and SCTC methods in patients with positive LA

Factors dosages performed with SYNT at three PD s in LA+ samples showed nonparallel DC in 36.7% of FVIII, 66.1% of FIX, and 86.2% of FXI.

There were 5/62 LA+ samples that showed FVIII SYNT 1/10 results < 50 IU/mL, all of them became > 50 IU/mL by FVIII SYNT 1/40, and the 62 presented normal or increased results by FVIII SCTC. Considering FIX SYNT 1/10 23/58 LA + samples analyzed presented values < 50 IU/mL, and 14/23 persisted low by FIX 1/40, being low by FIX SCT only 5/14 samples from patients under AVK treatment with INR > 3.0. In the analysis of FXI SYNT 1/10 18/30 LA+ samples presented results < 50 IU/mL and 8/18 persisted low by FXI SYNT 1/40, but all of them were normal by FXI SCTC.

Figure 1 shows all results from LA+ samples (both with parallel and nonparallel DC). In a smaller group of samples studied at the hemophilia center no statistically significant differences were found between FVIII SYNT, FVIII CHROM, and FVIII SCTC in NC and HA patients, nor in LA+ (only 4/15 presented nonparallel DC with FVIII SYNT), (Table 3 and Fig. 1).

Figure 2 shows bland altman graphs comparing FIX and FXI by SCTC and synth. In LA+ samples FIX SYNT 1/10 presented lower levels compared to FIX SYNT 1/40 and FIXSCTC. Even more, FIX SYNT 1/40 was lower than FIXSCTC (Table 2). This is clearly observed in Fig. 2C where some samples do not achieve normal values of FIX even at 1/40 dilution.

FXI SYNT 1/10 presented statistically lower results than FXI SYNT 1/40 and FXI SCTC in LA + samples, particularly those with non-parallel DC (Table 2, Fig. 2D).

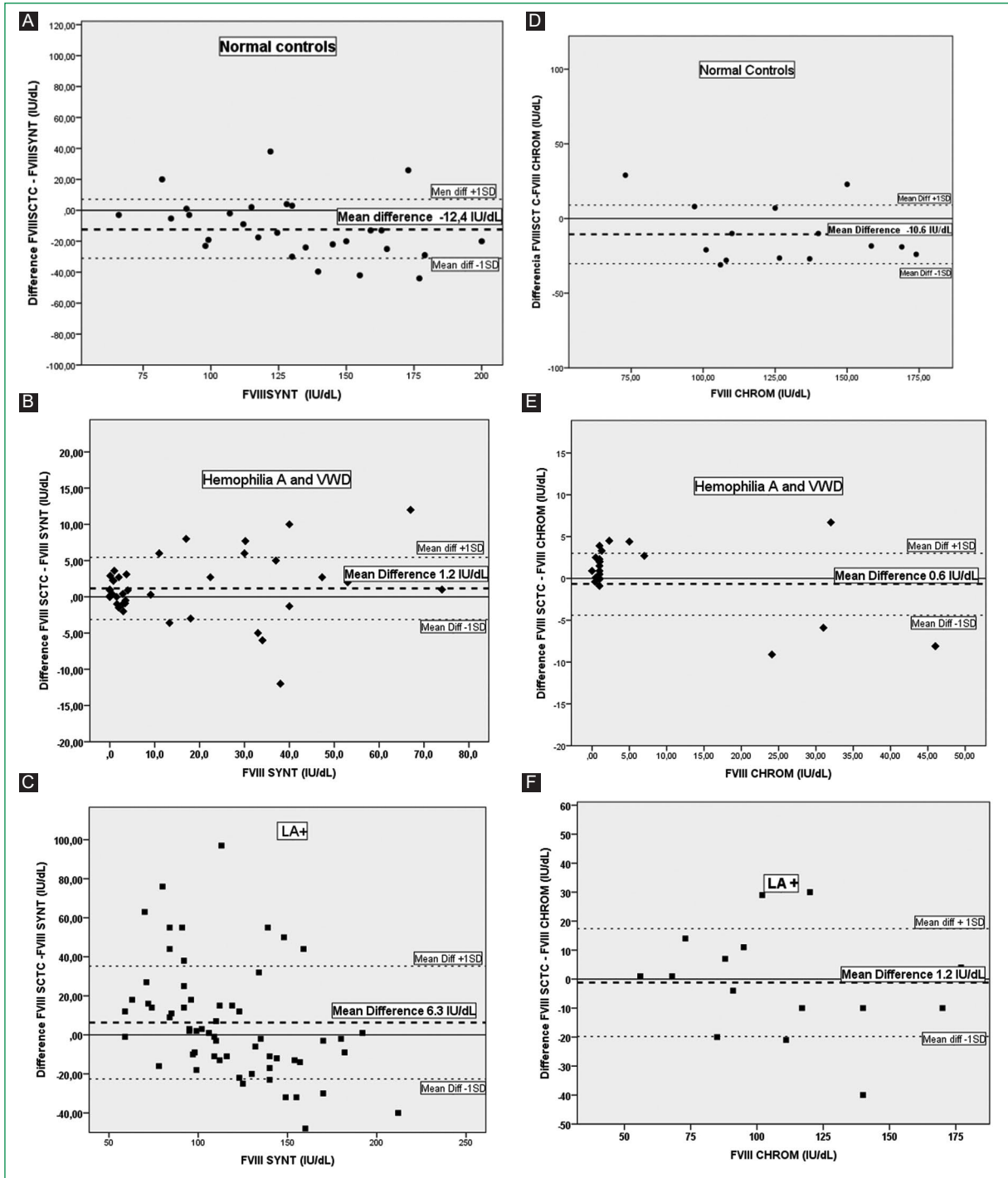


Figure 1. A: bland altman graphs comparing FVIII SICTC versus FVIII Synth in normal controls (NC), B: hemophilia A and von Willebrand Disease (VWD) patients, C: and lupus anticoagulant (LA) +, D: as well as FVIII SICTC versus FVIII CHROM in NC, E: hemophilia A and VWD patients and F: LA +.

Correlations between FVIII performed by SYNT and SCTC methods were better when using 1/40 PD than 1/10 PD in LA+ plasmas 0.759 versus 0.602 ($p < 0.001$ for both) but were really good between FVIII SICTC and FVIII: CHROM (0.949, $p < 0.001$). There was no

correlation between SCTC and SYNT 1/10 PD methods for FIX and only low statistical correlation with SYNT 1/40 PD (0.373, $p = 0.004$) in LA+ plasmas. Even worse, there was no correlation SYNT and SCTC methods in FXI measurements.

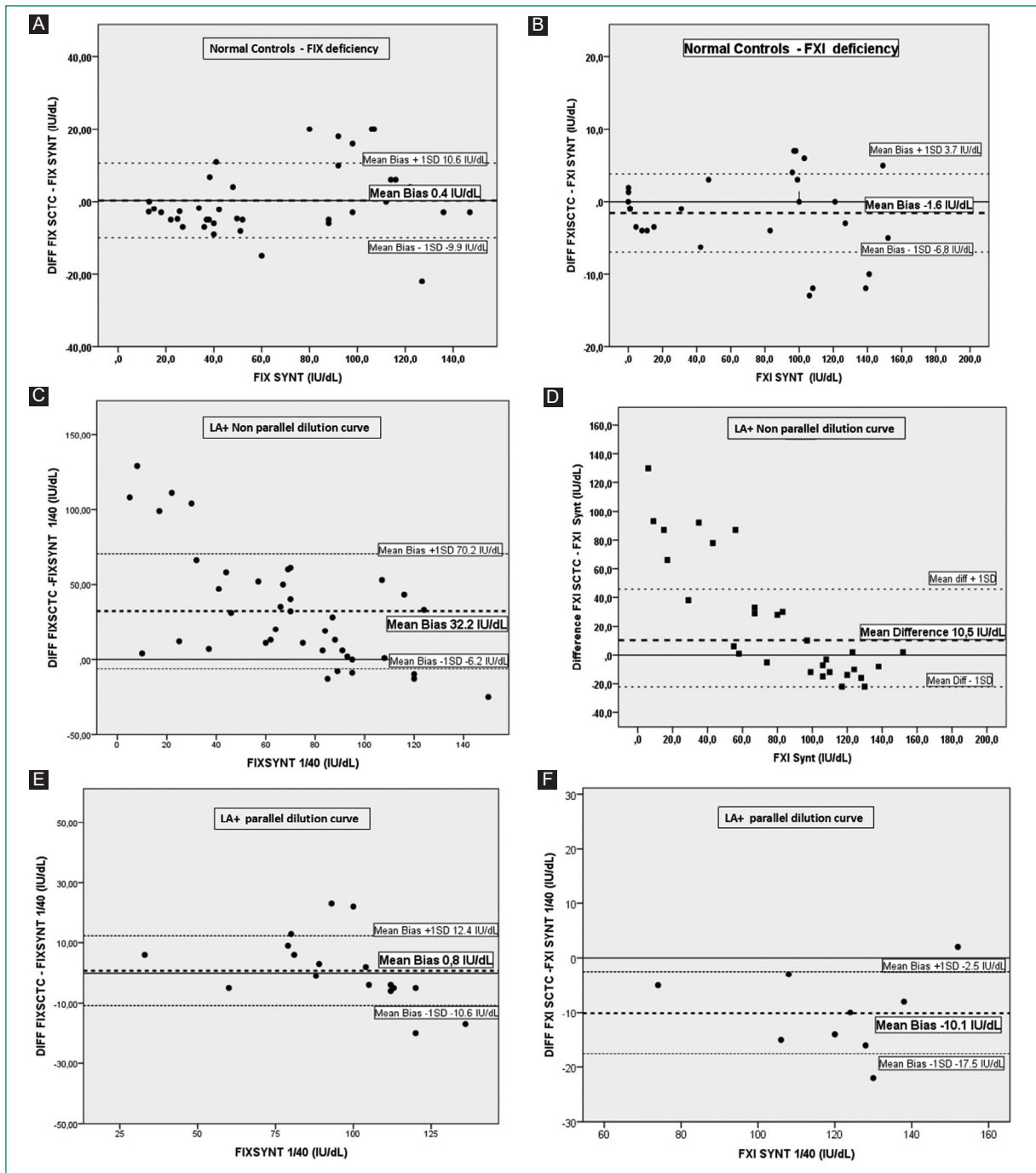


Figure 2. A: bland altman graphs comparing FIXSCTC versus FIX Synth in normal controls (NC) and FIX deficient, C: lupus anticoagulant (LA) + with non-parallel FIX Synth dilution curve (DC), E: and parallel FIX Synth DC, B: as well as FXISCTC versus FXI Synth in NC and FXI deficient, D: LA+ with non-parallel FXI Synth DC, F: and parallel FXI Synth DC.

In only two additional cases of patients presenting acquired HA and LA, coexisting both autoimmunity expressions, residual FVIII SCTC was higher than FVIII SYNT 1/40 PD, and in one patient, FVIII inhibitor titration was 1 BU lower with FVIII SCT than with FVIII SYNT.

Discussion

This study confirms that FVIII, FIX, and FXI levels could be measured with one stage clotting assay using an APTT reagent with silica as activator and high PL concentration (SCTC) in cases of LA + plasmas to

Table 3. FVIII measured by coagulometric standard (SYNT), alternative (SCTC) and chromogenic technics

Sample type	FVIII SYNT 1/10 Median (IQR)	FVIII SYNT 1/40 Median (IQR)	FVIII CHROM Median (IQR)	FVIII SCTC 1/10 Median (IQR)
Normal controls (n = 15)	126 (99-166)		126 (105-152)	108* (95-142)
LA + (n = 15)	101 (58-120)	109 (78-148)	102 (85-140)	95 (69-131)
FVIIID (n = 25)		2.9 (0.4-11.2)	1.0 (1.0-6.0)	3.0 (0.9-9.5)

*p = 0.046 FVIII SCT1/10 versus FVIII CHROM.
LA: lupus anticoagulant; FVIIID: FVIII deficient; IQR: interquartile range.

overcome LA interference and factor level underestimation avoiding results misinterpretation by clinicians. This is accordance with that previously published⁸ but in the present study, we used different standard APTT silica reagent and clot detection principle coagulometer. In addition, in the present study, a larger number of LA + and NC as well as FVIIID, FIXD, and FXID samples were tested which allowed to confirm that factor deficiencies can be correctly identify. It is important to consider that FVIII SCTL presented a very good correlation with FVIII CHROM which is considered the ideal method to overcome LA interference.

One interesting observation is that the no parallelism in LA+ samples was less frequent in the dosage of FVIII than FIX and FXI showed by many FIXSYNT and FXISYNT low results even at 1/40 PD. This agrees with the results from Tang and Yin⁹, who, in the 12 LA+ samples found BIAS < 20% less using APTT A compared to SCTC in only 2 samples for FVIII but in 5 for IX and 4 for FXI, even with higher PD. The reason for this finding is not known, but it could be thought that in intrinsic tenase complex the Gla Domain and Calcium-dependent anchorage of FIX on PL surface is very important, and PL concentration-dependent and LA compete with the binding of coagulation factors for catalytic PL surfaces in coagulation tests like APTT¹⁰. Considering that it has been demonstrated that FIX a has lower affinity (higher kinetic dissociation constant) to PL surfaces than FVIII a when forming the Tenase complex¹¹, FIX activity test could be more sensitive to the limited availability of anionic PL in the reaction when LA is present. Additionally, FVIII is an acute phase reactant, and its levels are frequently high in patients with autoimmune disease and thrombotic primary antiphospholipid syndrome, situations that could lessen the effect of LA in the FVIII assay¹².

The present study has limitations such as the fact that factor assays studied only up to 1/40 PD, a common procedure in clinical laboratories. As a strength, this

study included not only NCs and LA+ plasmas but evaluated plasmas from patients with congenital or acquired factor deficiencies to evaluate SCTC factors methods and even more FVIII SCTL was compared to FVIII CHROM.

Conclusion

FVIII, FIX, and FXI levels could be measured with one-stage clotting assay by using SCTC in LA+ patients to overcome LA interference and avoid factor levels underestimation. This method could be a reliable tool when deficiencies or specific factor inhibitors are suspected in a patient with LA, particularly when a CHROM test is not available.

Funding

None.

Conflicts of interest

None.

Ethical considerations

Protection of humans and animals. The authors declare that no experiments involving humans or animals were conducted for this research.

Confidentiality, informed consent, and ethical approval. The authors have obtained approval from the Ethics Committee for the analysis of routinely obtained and anonymized clinical data, so informed consent was not necessary. Relevant guidelines were followed.

Declaration on the use of artificial intelligence. The authors declare that no generative artificial intelligence was used in the writing of this manuscript.

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About treatment of Von Willebrand's disease in the 21st century

A propósito del tratamiento de la enfermedad de Von Willebrand en el siglo XXI

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Abstract

Von Willebrand disease is the most common bleeding disorder, often underdiagnosed, affecting both men and women. Diagnostic advances, including molecular biology, have improved detection. Traditional treatment is on-demand, but prophylaxis is gaining relevance, especially in severe or recurrent cases, following the hemophilia model. The latest guidelines recommend prophylaxis in patients with a history of severe and frequent bleeding, but evidence remains limited, supported by various retrospective studies. The PRO. WILL study demonstrated a significant reduction in the risk and frequency of bleeding with prophylaxis compared to on-demand treatment. Other studies support the efficacy of Wilate® and Veyvondi®, FVW/FVIII concentrates, although results vary depending on the type of von Willebrand disease and tolerability. More randomized controlled clinical trials are needed to better evaluate prophylaxis versus on-demand treatment, especially in mucosal bleeding and other scenarios.

Keywords: Von Willebrand disease. Prophylaxis. Hemophilia model. Retrospective studies. Randomized controlled trials.

Resumen

La enfermedad de Von Willebrand es la enfermedad hemorrágica más común, a menudo infradiagnosticada, que afecta tanto a hombres como a mujeres. Los avances diagnósticos, incluida la biología molecular, han mejorado su detección. El tratamiento tradicional es a demanda, pero la profilaxis está ganando relevancia, especialmente en casos graves o recurrentes, siguiendo el modelo de la hemofilia. Las últimas guías recomiendan la profilaxis en pacientes con antecedente de sangrado grave y frecuente, pero la evidencia sigue siendo limitada y apoyada en estudios retrospectivos. El estudio PRO. WILL demostró una reducción significativa del riesgo y de la frecuencia de sangrado con la profilaxis frente al tratamiento a demanda. Otros estudios respaldan la eficacia de Wilate® y Veyvondi®, concentrados de FVW/FVIII, aunque los resultados varían en cuanto al tipo de enfermedad de Von Willebrand y la tolerabilidad. Se necesitan más ensayos clínicos controlados aleatorizados para evaluar mejor la profilaxis en comparación con el tratamiento a demanda, en particular en sangrados de mucosas y otros escenarios.

Palabras clave: Enfermedad de Von Willebrand. Profilaxis. Modelo de hemofilia. Estudios retrospectivos. Ensayos clínicos controlados.

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Date of reception: 01-04-2024

Date of acceptance: 06-05-2024

DOI: 10.24875/RHT.M24000013

Available online: 18-09-2025

Hemost Trombos (Eng). 2025;2(2):68-72

www.revistahemostasiaytrombosis.com

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Introduction

Von Willebrand Disease (VWD) is the most common bleeding disorder worldwide, affecting men and women equally¹. It is estimated to have an incidence of 1% in the general population, although it is recognized that it may be underdiagnosed. In recent years, there have been significant advancements in diagnostic techniques, including molecular biology diagnostics, a field in which, in Spain, has demonstrated leadership with projects like PCM-EVW, led by Dr. Batlle Fonrrodona. The treatment of bleeding episodes remains primarily on-demand (OD), meaning the replacement of the deficient protein (in this case, von Willebrand factor) to stop bleeding when it occurs, with prophylaxis being less frequently used². However, recent clinical practice guidelines³ recommend its implementation in patients with severe or frequent bleeding phenotypes, especially since prophylaxis has become the reference treatment in patients with hemophilia A or B, with numerous guidelines from various scientific societies establishing recommendations for its implementation and follow-up. Given the hemorrhagic nature of VWD, it seems logical to follow a similar strategy in these patients to prevent bleeding and its consequences. Nonetheless, the documented experience on long-term prophylaxis in VWD is very limited and heterogeneous, highlighting the need for prospective clinical trials on the benefits of prophylaxis compared to OD treatment, particularly its benefit in mucosal bleeding³.

Discussion

Over the past 25 years, retrospective results have been reported regarding the use of prophylaxis as a treatment modality in patients with various types and subtypes of VWD, with different indications for initiating this therapeutic modality. In 2005, a Swedish experience⁴ compared OD treatment in 13 patients to prophylaxis in 35 patients, all with severe VWD (FVIII: C < 10%, VWF RiCo < 8%). In the prophylaxis group, with patients aged 3 to 65 years, the most frequent subtype was type 3 (28/35). Patients received a mean FVIII dose of 24 IU/kg (12-50) with a mean infusion frequency of 2.7 times per week (range, 1-3). Results suggest that long-term prophylaxis would be justified in most patients with type 3 VWD and other subtypes with severe bleeding tendencies and that such an approach could help avoid joint disease if started early.

A different study by Halimeh et al.⁵ reported the German experience of secondary prophylaxis in 32 patients with

VWD (13 adults, 7 adolescents, and 12 pediatric patients), with a median follow-up of 3 years (range, 1-9). The most common subtype was type 2 (n = 15), followed by type 3 (n = 14) and type 1 (n = 4). Patients were treated with Humate-P® (n = 8, 1 switched to Wilfact®) or Wilate® (n = 24) and administered a median dose of 40 IU VWF RiCo/kg, with an infusion frequency varying between 3 times/week (n = 7), 2/week (n = 23), and 4/week (2 children). The decision to initiate prophylaxis was based on a bleeding score > 2 before diagnosis, concomitant with recurrent bleeding associated with anemia in patients with OD treatment for VWD. Within 12 months, hemoglobin concentrations returned to normal values, and recurrent bleeding ceased in 31 of 32 patients, with 1 patient developing inhibitor antibodies. After a 12-month observation period, monthly bleeding frequency and bleeding score were significantly reduced (3 vs. 0.07; 3 vs. 0; p < 0.001) vs. pre-prophylaxis and pre-diagnosis values, which led authors to conclude that secondary prophylaxis is effective, well-tolerated, and highly beneficial for patients with recurrent bleeding.

The publication of the Von Willebrand Disease Network⁶ in 2012 highlighted the importance of having registries to reliably document or evaluate the type and frequency of bleeding before and after the initiation of prophylaxis in these patients. Regarding the obtained results, it concluded that prophylaxis in VWD was effective considering the annualized bleeding rates for the period before prophylaxis, during prophylaxis, and for primary bleeding indication, defined as the site that presented more than 50% of all bleeding episodes. The work included 61 patients from 20 centers in 10 countries, and for analytical purposes, a total of 59 subjects were considered. The mean age at the start of prophylaxis was 22.4 years. The most represented subtype was type 3 (n = 34, 57.6%). The intra-patient bleeding rates during prophylaxis vs. pre-prophylaxis were significantly different for the entire group (p < 0.0001) and for those with primary bleeding indications due to epistaxis (p = 0.0005), joint bleeding (p = 0.002), and GI bleeding (p = 0.001). Additionally, the benefit of prophylaxis was independent of age (< 18 years vs. > 18 years).

A Swedish study⁷ published in 2018 retrospectively evaluated the impact of VWD on hospitalization frequency vs. a control group and whether prophylaxis was associated with fewer admissions, generally due to GI bleeding, menorrhagia, and epistaxis. It concluded that subjects with VWD had a considerably higher risk of health care resource consumption vs. controls, and that the initiation of prophylaxis could reduce the number of bleeding-related admissions.

The PRO.WILL study⁸ is an open-label, phase III, 12-month study in which patients with severe VWD were randomized to receive PROPHYLAXIS (n = 9; 5 completed) or OD (n = 10; 7 completed) with a single product (Fandhi®/Alphanate®, Grifols Lab), evaluating the proportion of patients who did not present any spontaneous bleeding episode, adverse events (AE), or thrombotic events. All OD patients had type 2 or 3 VWD vs. 70% of patients on prophylaxis. The mean dose used was 38.6 IU FVIII: C/kg every 3 days (n = 2) or on alternate days (n = 3). All OD patients experienced spontaneous bleeding vs. 60% of patients on prophylaxis who showed fewer bleeding episodes (n = 32 vs. n = 172 [112 in the same patient, mainly mucocutaneous]; p < 0.0001) and lower bleeding risk (estimated attributable risk, -0.667; 95%CI, -2.374, -0.107; p < 0.001). The most common bleeding episodes in the OD and prophylaxis groups were, respectively, epistaxis (n = 52 vs. n = 15) and GI (n = 1 vs. n = 13 [9 in the same patient]). While most bleeding episodes lasted 1 day in the OD group (31/32), in the prophylaxis group, only epistaxis did (14/15). Additionally, the probability of remaining bleed-free over time was higher in patients on prophylaxis vs. OD patients, stabilizing in the prophylaxis group approximately 2 months after initiation. Thus, despite the small sample size and heterogeneity of the study population, patients on prophylaxis with Fandhi®/Alphanate® had lower risk and bleeding rates vs. OD treatment.

In 2020, the results of a prospective, observational, post-marketing study of another plasma-derived VWF concentrate containing only VWF (Wilfactin®) were reported, in relation to the treatment of VWD. A total of 155 out of 174 enrolled patients from 31 centers were eligible for efficacy evaluation, 76.8% of whom were severely affected (VWF: RiCo ≤ 15 IU/dL). In 32 patients on prophylaxis, the median dose per infusion was 43.5 IU VWF/kg, administered approximately 2x/week. Bleeding occurred in 1.5% of all infusions, and the median ABR was 1.0 for 20 patients treated ≥ 12 months. Moreover, tolerability was excellent, with no safety issues or thrombotic events⁹.

The latest clinical practice guidelines on the management of VWD³, resulting from the collaboration of various scientific societies, published in 2021, suggest long-term prophylaxis over no prophylaxis in patients with VWD with a history of frequent and severe bleeding, although it should be periodically reviewed, and they highlight the low certainty regarding the existing evidence of its positive effects. The same clinical guidelines identify a series of additional needs for

further research, such as the need for large, randomized controlled clinical trials comparing continuous prophylaxis with OD treatment, particularly in patients with mucosal bleeding, studies on prophylaxis for heavy menstrual bleeding, GI bleeding, or the impact of prophylaxis on quality of life, and the need for studies on the use of plasma-derived VWF (pdVWF) concentrate vs. recombinant origin concentrate, among others.

In this regard, in 2024, Sidonio et al.¹⁰ published the results of the WIL-31 study, the first phase 3, prospective, uncontrolled trial that evaluated the safety and efficacy profile of Wilate® prophylaxis over 12 months in male and female patients ≥ 6 years old with VWD with severe or frequent bleeding phenotype (more than 6 spontaneous bleeding episodes (SBEs) during OD treatment and, at least, 2 SBEs requiring treatment with VWF concentrates), except type 2N, who had previously completed a 6-month prospective OD study (WIL-29)⁸. The median age of the patients (n = 33) was 18 years, and the most represented VWD type was type 3 (67%), followed by type 2 (15%). The primary endpoint was a > 50% reduction in the mean total annualized bleeding rate (ABR) with Wilate® prophylaxis vs. previous OD treatment. During prophylaxis, the total ABR was 5.2 (84.4% reduction), with the reduction consistent by age, sex, and VWD type. Additionally, the mean spontaneous ABR was reduced by 86.9% (mean, 3.2) vs. OD treatment, with 10 (30.3%) and 15 (45.5%) patients experiencing no bleeding during the 12 months of prophylaxis, and of the 173 reported bleeding episodes, 84.4% were categorized as mild, and 69.9% were treated. Furthermore, 2 aspects are noteworthy: no serious treatment-related adverse events or thrombotic episodes were reported, and the study globally demonstrated that Wilate® prophylaxis was effective and well-tolerated in pediatric and adult patients with VWD of all types.

Wilate®¹¹ is a plasma-derived VWF/FVIII concentrate with a 1:1 VWF/FVIII ratio, offering an advantage in terms of laboratory monitoring, which can be performed by determining F8 levels. Additionally, given the 1:1 VWF/FVIII ratio, the clearance of both molecules occurs in parallel, more predictably, avoiding FVIII accumulation and thus the thrombotic risk associated with high FVIII levels (≥ 150%). Furthermore, during its manufacturing process, it undergoes double viral inactivation according to European Medicines Agency (EMA) standards¹², leading some authors¹³ to state that the experience gained in recent decades has resulted in the absence of pathogen transmission by the current generation of plasma derivatives, making them the

safest plasma-derived concentrates in history, with no reported cases of viral infection transmission in the past 20 years. On the other hand, unlike hemophilia, efforts to obtain an approved recombinant VWF (Veyvondi®, Takeda Lab)¹⁴ are pending price reimbursement in Spain, licensed by the EMA for prophylaxis and treatment of bleeding and surgical bleeding prophylaxis. However, for prophylactic treatment, the product was only approved by the FDA for type 3 VWD patients previously on OD treatment. In the study published by Leebeck et al.¹⁵ in 2022, 10 patients on previous prophylaxis with pdVWF were included, and the results were compared with those obtained after switching to rVWF prophylaxis: the authors found similar results to those obtained with Fandhi®/Alphanate® prophylaxis in the PRO.WILL study, with factor consumption, in IU VWF RiCo/kg per infusion (approximately 50), and similar weekly infusion frequency (2/week). However, this study has the significant limitation of being neither randomized nor comparative and including a limited number of patients. In 2023, the same authors, in a post hoc analysis¹⁶, reported a reduction in spontaneous and traumatic ABR in 45% of patients after switching from pdVWF to Veyvondi®, although there was no improvement in spontaneous and treated ABR in 45% of patients; in fact, prior to the switch (Table 1), 62.5% of patients on pdVWF prophylaxis experienced 2 or fewer bleeding episodes, and after switching to Veyvondi®, the percentage of patients with 2 or more bleeding episodes increased from 37.5% up to 62.5%, suggesting no net benefit from switching products. Therefore, while Veyvondi® prophylaxis reduces treated spontaneous ABR vs. previous OD treatment, it merely maintains similar hemostatic control in patients on prophylaxis after switching from prior pdVWF. One notable aspect of this study is the inclusion of patients on previous OD treatment who had experienced 3 or more spontaneous bleeding episodes in the previous 12 months, excluding menorrhagia. In light of the WIL-31 trial results, the real paradigm shift may come from increased implementation of pdVWF prophylaxis—Wilate® in this case—in patients with severe or recurrent bleeding phenotypes. Additionally, in certain patients and scenarios, the use of Veyvondi® requires the concomitant administration of a FVIII dose in patients with baseline F8 levels < 40% or if unknown, in situations requiring rapid hemostasis normalization, such as acute bleeding, severe trauma, or emergency surgery. Hancock and Escobar¹⁷ assert that this new concentrate has greater hemostatic potential, possibly due to the presence of ultra-high VWF multimers and

Table 1. Change in bleeding frequency after switching to Veyvondi®

Bleeding events	pdVWF	SWITCH	Veyvondi®
0	25%		37,5%
> 0-2	37,5%		0
> 2-5	25% 37,5%		25% 62,5%
> 5	12,5%		37,5%

Adapted from Leebeck et al.¹⁵

a more favorable high molecular weight multimer pattern vs. previous pdVWF. This claim contrasts with the median weekly VWF consumption reported in both studies related to prophylaxis, which for patients with VWD on Wilate® was 58 IU/kg (28.2-114) (n = 33) and 95.6 IU/kg (34.9-187.8) with Veyvondi® (n = 30), practically over 40% consumption. Furthermore, the benefit of the presence of these multimers is unclear once their accumulation, in plasma and on endothelial cell surfaces, due to deficiency or abnormality in ADAMTS13, can induce platelet aggregation and adhesion to the vascular endothelium, associated with thrombotic microangiopathies, inflammation, or microcirculatory thrombosis, such as acute myocardial infarction or sepsis-induced disseminated intravascular coagulation, among others¹⁸. Indeed, unlike the WIL-31 study, a thromboembolic event was reported in a patient. Regarding viral safety, it is known that recombinant products—such as Veyvondi®, obtained from a CHO cell line—could theoretically transmit human or non-human mammalian viruses introduced through master cell lines or human or animal proteins contained in the medium used for cell freezing or culture¹⁹, which is why, as previously mentioned, they must undergo the same viral inactivation processes as plasma-derived concentrates¹².

Conclusions

In conclusion, after years of retrospective studies highlighting the potential benefits of prophylaxis in VWD, but without achieving the results seen in hemophilia, although with a marked reduction in ABR, the results of the WIL-31 trial, with Wilate® or the clinical trial of Veyvondi® seem to justify the implementation of prophylaxis in patients with VWD of all types, any age, and both sexes, with a marked reduction in bleeding. It could be said that pdVWF, such as Wilate®, has demonstrated high efficacy from a hemostatic point of view, in various scenarios, whether for bleeding

treatment or surgical bleeding prophylaxis, as evidenced in the WIL-31 trial for prophylaxis. Additionally, from a viral safety perspective, we are dealing with the safest plasma-derived concentrates ever, as evidenced by the reported figures in the UK, where in 2022 and 2023, 68,892,532 IU of VWF were uneventfully transfused in 1034 patients from an infectious standpoint²⁰. The possibility of infection by hepatitis B and C viruses, or HIV, along with hepatitis A and B vaccination, is currently more theoretical with the current measures implemented by manufacturers²¹. Thanks to these improved and thorough pathogen reduction steps, physicians and patients should trust not only the well-demonstrated efficacy but also on the safety of these plasma-derived concentrates²² for indications such as VWD treatment, as has been done until now. This is crucial when, with the advent of recombinant products and in light of published studies, it is not clear that these products offer any real net benefit, at least, in terms of efficacy and probably efficiency, at a time when the clinical benefit of prophylaxis adoption in VWD is finally being prospectively reported, at least, in some patient subgroups and for certain types of bleeding.

Increased use of prophylaxis will impact health care system costs, and after observing that switching from previous pdVWF prophylaxis to a recombinant product does not seem to improve the bleeding pattern but rather maintains similar efficacy, as recognized by the authors, we should be cautious in recommending one product over another.

Funding

None.

Conflicts of interest

None.

Ethical considerations

Protection of humans and animals. The authors declare that no experiments involving humans or animals were conducted for this research.

Confidentiality, informed consent, and ethical approval. The study does not involve patient personal

data nor requires ethical approval. The SAGER guidelines do not apply.

Declaration on the use of artificial intelligence.

The authors declare that no generative artificial intelligence was used in the writing of this manuscript.

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Switch from extended half-life factor VIII to emicizumab in a poorly controlled hemophilia A patient: a case report

Intercambio de factor VIII de vida media extendida a emicizumab en un paciente con hemofilia A mal controlada: reporte de caso

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We present the case of a 20-year-old male diagnosed at 6 months of age with severe hemophilia A (mutation c.670+5G>A), with no family history of the disease except for bleeding episodes in his mother and no history of factor VIII inhibitors. The patient had generally good joint health and led a sedentary lifestyle.

Primary prophylaxis was initiated with Advate® (octocog alfa, SHL factor) 3 times/week at a dose of 1250-1250-1500 IU (Monday, Wednesday, Friday) (Table 1), adjusted according to body weight and clinical response. In December 2014, at 9 years of age, the patient was enrolled in a pediatric clinical trial with Adynovi® (rurioctocog alfa pegol, extended half-life [EHL]), receiving a dose of 50 IU/kg twice weekly (39 kg, 2000 IU/twice weekly). During this period, disease control was well maintained, with no bleeding episodes.

A few years later, in February 2018, an alternative therapeutic approach needed to be considered, as the clinical trial ended and the European Medicines Agency authorization¹ did not support the use of Adynovi® (pegylated EHL) in children under 12 years old due to the risk of vacuolation with prolonged polyethylene glycol (PEG) exposure.

As the patient had shown a favorable response to Adynovi®, the decision was made to switch to another non-pegylated EHL factor, Elocta®, which was approved for pediatric use. Treatment was initiated at 2000 IU twice weekly; however, the appearance of subcutaneous hematomas, along with low trough levels (0.5% at day 3), led to an upward dose adjustment to 3000 IU while maintaining the dosing interval. Despite this adjustment, trough levels remained low. Given that the patient had surpassed the age threshold of 12 years, at 14 years of age, Adynovi® was reintroduced at 3000 IU twice weekly. Although the half-life improved (11.75 h), estimated trough levels remained low (Table 1). Nevertheless, the patient did not experience any bleeding complications during the 1st year of treatment and responded well to a minor surgical procedure (myringotomy with tympanic drainage) without requiring additional FVIII doses.

In 2019, the patient developed joint limitations in the right knee with an insidious onset, unrelated to any specific trauma. Edema began 12 h after administration of a 3000 IU Adynovi® dose, which was being administered every 2 days at that time. Upon examination at 24 h, no signs of swelling or

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Date of reception: 25-03-2025

Date of acceptance: 23-05-2025

DOI: 10.24875/RHT.25000009

Available online: 18-09-2025

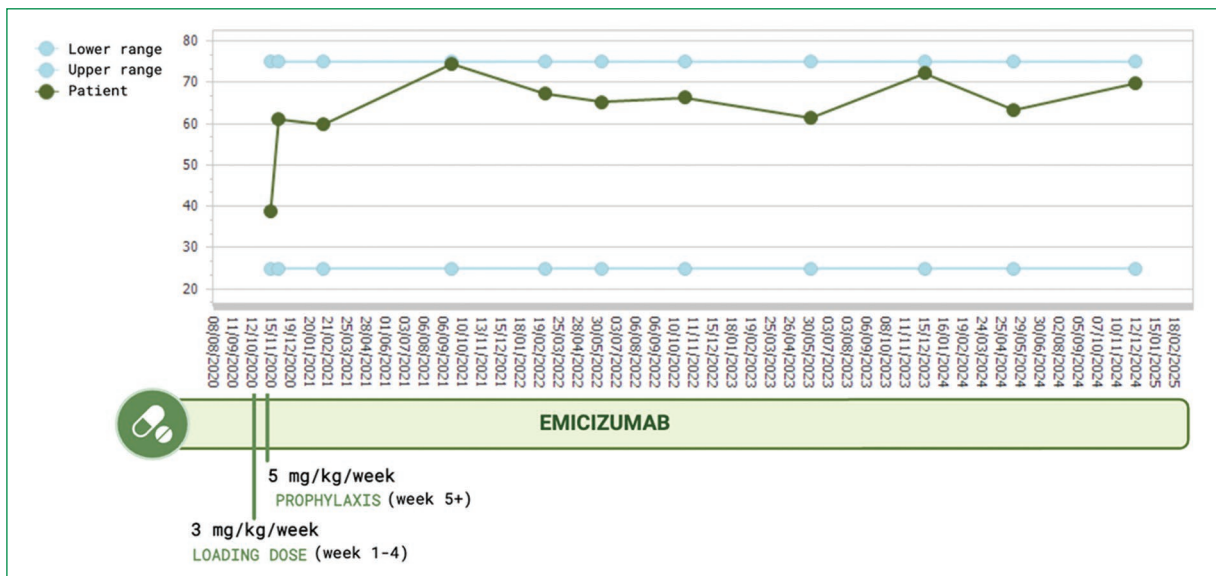
Hemost Trombos (Eng). 2025;2(2):73-76

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Table 1. Comparison of the pharmacokinetic parameters obtained with each FVIII

Variables	Maintenance regimen before hemarthrosis			Intensified regimen after hemarthrosis	
	rFVIII (Advate®)	rFVIII-Fc (Elocta®)	PEG-rFVIII (Adynovi®)	PEG-rFVIII (Adynovi®)	rFVIII-Fc (Elocta®)
PK date	November 17, 2015	April 10, 2018	March 20, 2019	March 04, 2020	September 25, 2020
Regimen (IU)	1250-1250-1500 MWF	3000 TF	3000 TF	4000/24 h	4000/24 h
N° weekly doses	3	2	2	7	7
Dose/kg/week	111.1	105.3	105.3	388.9	388.9
t1/2 (h)	8	10	11.8	7.75	8.5
Peak (IU/dL)	81	112	125	153	170
AUC (IU*h)/L	6214	8980.3	17810.5	ND	ND
Trough 24 h (IU/dL)	6.3	12.7	23.7	14.7	17.8
Trough 48 h (IU/dL)	1.2	3.2	6	2.1	2.9
Trough 72 h (IU/dL)	0.5	1.3	1.8	0.6	0.8
T5% (h)	26.8	39.3	51.5	36.8	40.3
T2% (h)	39.3	58.3	69.5	48.8	53.5
T1% (h)	51.3	93	87	60.3	66

AUC: area under the curve; M: monday, ND: not determined; T: tuesday; T5%, 2%, 1%: time to 5%, 2%, 1% Activity W: wednesday; F: friday.

**Figure 1.** Plasma concentration of emicizumab.

tenderness on palpation were observed. However, serial ultrasound monitoring of the affected right knee was conducted in the days following the event. The ultrasound findings revealed abundant synovial fluid, consistent with hemarthrosis, leading to

arthrocentesis, which yielded more than 25 mL of joint fluid. The fluid persisted with erratic thickness variations of 5.5, 8.5, and 6 mm at days 4, 9, and 11, respectively. Infection was ruled out, as joint fluid cultures were negative.

To manage the condition, trough levels were measured at 24 h, yielding 11.4 IU/dL, and a pharmacokinetic (PK) study revealed a reduction in Adynovi®'s biological half-life (7.75 h). Consequently, to treat the active knee hemarthrosis, a regimen of 3000 IU/24 h was initiated, which was later increased to 4000 IU/24 h due to the slow resolution of the hemarthrosis and poor response with low trough levels (13.4% at 24 h) despite high doses. These findings, along with the suspicion of anti-PEG antibody generation (despite the fact that they were ultimately not detected), led to the discontinuation of Adynovi® and a switch back to Elocta® while maintaining the same dosing regimen. The estimated half-life with Elocta® was 11.0 h, allowing for an extension of the dosing interval to every 48 h. Following the resolution of hemarthrosis after 2½ months, the regimen was further extended to every 72 h.

Despite the seemingly favorable clinical course, PK estimations showed another decline in half-life (8.5 h), with trough levels < 1% at 72 h. Given the erratic PK profile, the high treatment burden, and the substantial FVIII consumption, various therapeutic options were considered, ultimately leading to the proposal of a non-replacement therapy: emicizumab.

In October 2020, the decision was made to initiate emicizumab (Hemlibra®), considering the advantages of subcutaneous administration and its simplified dosing schedule². The loading dose regimen of 3 mg/kg/week (210 mg, two vials of 105 mg) was administered for 4 weeks, while Elocta® 4000 IU was continued 3 times/week during the 1st week of the transition. The maintenance regimen of 1.5 mg/kg/week (one vial of 105 mg) was then initiated, completing 1 year of treatment without any bleeding episodes or requiring additional FVIII doses. The annual treatment cost was reduced from €225,600 (considering the highest FVIII prophylaxis regimen: 4000 IU/72 h of Esperoct®) to €153,765 with Hemlibra®, leading to total annual savings of €71,835.

Emicizumab levels were monitored from treatment initiation (10/30/2020) to the present (Fig. 1), with mean $\pm$ standard deviation (SD) levels of 63.6 ± 9.5 μ g/mL (min: 38.7, max: 74.5 μ g/mL). The patient continues on the same weekly 105 mg dose, with optimal bleeding control and no reported treatment-related adverse reactions, including at the injection site.

This clinical case illustrates the complexity of therapeutic management in patients with severe hemophilia A, highlighting the importance of guided PK monitoring and individualized treatment adaptation.

The variability in the patient's response to factor VIII (FVIII) underscores the need for a personalized approach, supported by advanced tools such as the WAPPS-Hemo platform³, which enables precise PK estimations and objective data-driven therapeutic adjustments.

The decision to initiate emicizumab (Hemlibra®) marked a turning point in the patient's management. The patient achieved optimal bleeding control, with no hemorrhagic episodes or additional FVIII requirements during the 1st year of treatment. This approach not only improved hemostatic control but also reduced the risk of joint complications and excessive FVIII consumption, aligning with published literature⁴ and resulting in significant economic implications.

The levels observed in this patient (mean $\pm$ SD: 63.6 ± 9.5 μ g/mL) remained within the range reported in clinical trials (95% confidence interval: 25–80 μ g/mL)^{5,6}, with minimal fluctuation (Interquartile range: 61.2–68.5), suggesting an adequate response. However, no clearly established therapeutic levels currently exist. Few studies have addressed this need, although Donners et al. propose a target emicizumab level of 30 μ g/mL, as no differences in bleeding control were observed at higher concentrations⁷.

At present, emicizumab does not require PK-guided therapeutic monitoring², primarily due to its stable PK profile over time, which remains consistent in both adult and pediatric patients and across all dosing regimens (1.5 mg/kg/week, 3 mg/kg/2 weeks, and 6 mg/kg/4 weeks)^{5,6}. Nevertheless, PK-guided monitoring could be of interest in complex clinical situations, such as managing spontaneous bleeding in combined treatment with emicizumab and FVIII. In this context, hospital pharmacists have a valuable opportunity to optimize patient management and investigate the correlation between specific target levels and clinical efficacy.

Funding

None.

Conflicts of interest

None.

Ethical considerations

Protection of humans and animals. The authors declare that the procedures followed complied with the

ethical standards of the responsible human experimentation committee and adhered to the World Medical Association and the Declaration of Helsinki. The procedures were approved by the institutional Ethics Committee.

Confidentiality, informed consent, and ethical approval. The authors have followed their institution's confidentiality protocols, obtained informed consent from patients, and received approval from the Ethics Committee. The SAGER guidelines were followed according to the nature of the study.

Declaration on the use of artificial intelligence. The authors declare that no generative artificial intelligence was used in the writing of this manuscript.

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Anti-emicizumab antibodies in severe hemophilia A: report of two cases

Anticuerpos antiemicizumab en hemofilia A grave: reporte de dos casos

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Introduction

Hemophilia A is an inherited bleeding disorder characterized by factor VIII (FVIII) deficiency¹. Emicizumab (Hemlibra®, Roche) is a humanized bispecific monoclonal antibody that has been approved for prophylaxis in subjects with hemophilia A, with and without inhibitors, and has been proven safe and effective in preventing bleeding in HAVEN clinical trials^{2,3}. Fortunately, anti-emicizumab antibodies (AEAs) are very rare, and in the HAVEN clinical trials, only 3 out of 698 patients developed AEAs with neutralizing potential⁴. Identifying these AEAs is an important aspect in the clinical management of patients on emicizumab. In this article, we describe 2 cases of patients with AEAs, analyzing the clinical and lab test results. The patients come from 2 different centers across different countries: case report #1 comes from Argentina and case report #2 from Uruguay. In the Argentine center, 35 hemophilia patients up to 15 years old are treated. The multidisciplinary team uses the World Federation of Hemophilia clinical practice guidelines to establish primary and secondary criteria for prophylaxis; 95% of patients are treated with primary prophylaxis. Five patients have anti-FVIII inhibitors (IFVIII), 4 of whom are treated with emicizumab, 3 of whom failed previous immune

tolerance. The choice of emicizumab treatment is motivated by the fact that patients stop bleeding and improve their quality of life. Another issue is that all of them live > 100 km from the health center. In Uruguay, at Hospital Pereira Rossell, 100 hemophilia patients younger than 18 years are treated, and primary prophylaxis is performed in 100% of patients with severe hemophilia, as well as in moderate and mild patients with a bleeding phenotype. A total of 58 patients with severe hemophilia A are treated, 55 of whom do not have inhibitors and 3 do. All 58 patients are on emicizumab because they have severe hemophilia A, an indication by the Fondo Nacional de Recursos del Uruguay.

Case report #1

This is a 3-year-old boy with no family history of hemophilia. At 3 days of life, he presented a large hematoma on his back, requiring admission to the pediatric intensive care unit and eventually mechanical ventilation. He was diagnosed with severe hemophilia A and started recombinant FVIII treatment, with favorable clinical resolution. At 15 months, due to his bleeding phenotype, he started prophylaxis with recombinant FVIII. In the Argentine center, IFVIII is tested every 5 applications within the first 20, and then every

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Date of reception: 24-01-2025
Date of acceptance: 11-06-2025
DOI: 10.24875/RHT.M25000045

Available online: 18-09-2025
Hemost Trombos (Eng). 2025;2(2):77-79
www.revistahemostasiaytrombosis.com

Table 1. Lab test results

Case	Age (years)	Weeks into therapy	aPTT (S)	FVIII OSA (IU/dL)	FVIII CSA (IU/dL)	IFVIII (BU)	EMI (µg/mL)	AEA (BU)
A	3	29	98	< 1	< 1	281	ND	3.6
B	4	44	91	< 1	< 1	< 0.6	ND	5.0

ND: non detectable.

6 months. During applications 15 to 16, the appearance of an inhibitor was suspected due to hemorrhagic episodes, and the lab test results confirmed the clinical suspicion 6 weeks into therapy, reporting an IFVIII of 281 Bethesda units. He then began a regimen of 3 mg/kg emicizumab prophylaxis every 2 weeks, with good clinical response. Seven months into emicizumab therapy, he sustained a blow to the forehead after falling from a height of 50 cm, requiring hospitalization. The hospital lab test results reported a normal prothrombin time and a very prolonged activated partial thromboplastin time (aPTT) of 85 seconds. Adherence to treatment was very good, suggesting that the prolonged aPTT could be due to a loss of emicizumab effect. Samples were sent to a higher complexity laboratory, which reported undetectable plasma emicizumab and the presence of a neutralizing emicizumab inhibitor (Table 1). Since the last event, the boy continues to present new hematomas and occasional minor bleeding events. Weekly doses of 1.5 mg/kg were, then, decided. With this new prescription, the boy presented clinical improvement, with no changes in laboratory parameters and only occasional hematomas in the lower limbs, suggesting continued interference with emicizumab action. Recombinant activated factor VII was considered as a therapeutic option for eventual bleeding, and prophylaxis with other non-replacement therapies indicated for hemophilia A with inhibitors.

Case report #2

Case report #2 is a 4-year-old boy diagnosed with severe hemophilia A. Family history: maternal uncle with severe hemophilia A. The patient started emicizumab prophylaxis at 3 years of age, with a regimen of 6 mg/kg for 4 weeks. He has not experienced any adverse reactions associated with the treatment and does not present target joints. Annual controls have not shown reactive serology for sexually transmitted infections or the development of IFVIII. He receives FVIII, 500 IU every 28 days, as a tolerization regimen. In the

Uruguayan center, inhibitors are systematically tested every 6 months and always preoperatively or when there is a failure of appropriate hemostatic treatment. In the context of a semi-annual control, having received 10 FVIII exposures and at a maintenance dose of emicizumab, lab test results showed a very prolonged aPTT of 98 seconds, with normal coagulation parameters. Physical examination revealed no ecchymosis, hematomas, or hemarthrosis. Adherence to treatment is very good, as the boy participates in a patient assistance program with a nurse who administers the drug, suggesting that the prolonged aPTT may be due to some interference with emicizumab. Samples were sent to a higher complexity laboratory, which reported undetectable plasma emicizumab, negative IFVIII, and the presence of a neutralizing emicizumab inhibitor (Table 1). Since then, the boy has remained free of hemarthrosis or spontaneous or traumatic muscular hematomas or bleeding in other sites. Emicizumab and FVIII 500 IU continue as a tolerization regimen, with more frequent clinical controls.

Laboratory evaluation

Plasma FVIII levels were determined with a 1-stage coagulation assay (OSA) using Actin FSL® (Siemens) aPTT reagent, FVIII-deficient plasma (STA deficient plasma, Stago) plus a chromogenic assay (CSA) based on bovine coagulation factors (FVIII Chromogenic Assay®, Siemens). To measure plasma emicizumab concentration, we used a modified FVIII OSA assay calibrated with a specific emicizumab calibrator (in-house) and FVIII-deficient plasma (STA Deficient Plasma, Stago), and measured FVIII inhibitor activity with the CSA using the Nijmegen-Bethesda chromogenic assay. To prepare the emicizumab calibrator, we prepared a standard solution 1 (SS1) from an aliquot of commercial product (30,000 µg/mL) by diluting emicizumab 1/10 in imidazole buffer at pH 7.35 (3000 µg/mL). Then, a second standard solution (SS2) was prepared by diluting 1/10 of SS1 in commercial FVIII deficient plasma (300 µg/mL). We prepared each point of the calibration curve by diluting SS2

in FVIIIId to obtain the following concentrations: 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, and 6.25 µg/mL.

In a modified version of the Bethesda assay to measure AEAs, we added equal parts of patient plasma (and patient dilutions with dilution buffer, Owren-Koller®, Stago) and plasma from a patient with a known concentration of emicizumab to each tube. After incubation for 2 hours at 37 °C, each sample was analyzed by OSA for FVIII and emicizumab activity. The resulting values were, then, used to calculate the inhibitor titer expressed in Bethesda units. All coagulometric and chromogenic assays were performed on the STA Evolution Max2 (Stago) coagulometer.

Discussion

Emicizumab is a highly effective agent with minimal immunogenicity⁵. It's estimated that around 25,000 patients have received emicizumab to date, and case reports are very few⁶⁻⁹, making the development of AEAs a very rare event. AEAs can reduce drug efficacy via 2 main mechanisms: one is by directly blocking and interfering with the drug's active site on its target, and the other is by accelerating its clearance¹⁰. In the described cases, we used the modified Bethesda method to evaluate the effect of emicizumab and detected neutralizing activity vs emicizumab in both patients. While AEAs are rare, cases with clinical bleeding have been reported, making early diagnosis crucial. Although unexpected bleeding, which suggests therapeutic failure, is a relevant clinical sign, it may not always be present, as seen in case report #2. Regardless of the presence of bleeding, detecting these neutralizing antibodies is vital due to their impact on future clinical management decisions. From a laboratory monitoring perspective, the appearance of AEAs can be suspected when a prolonged aPTT is observed, as happened in the described cases. However, this detection method may not be adequate in the presence of low-titer neutralizing or non-neutralizing antibodies, which are only detectable by sensitive immunoassays⁷. Another important feature for clinical and laboratory evaluation is that AEAs reported in other case series were identified within the first into therapy, which is similar to our 2 patients. Additionally, they can appear, as in case report #2, in patients without anti-FVIII inhibitors (IFVIII)⁸. More reports will be essential to help clinicians make decisions with the best available evidence.

Funding

None declared.

Conflicts of interest

None declared.

Ethical considerations

Protection of persons and animals. The authors declare that no experiments on humans or animals were conducted for this research.

Confidentiality, informed consent, and ethical approval. The authors have followed the confidentiality protocols of their institution, have obtained informed consent from the patients, and have the approval of the Ethics Committee. The recommendations of the SAGER guidelines have been followed, according to the nature of the study.

Statement on the use of artificial intelligence. The authors declare that they did not use any type of generative artificial intelligence for the writing of this manuscript.

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