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Measuring Thrombus Stability at High Shear, Together With Thrombus Formation and Endogenous Fibrinolysis: First Experience Using the Global Thrombosis Test 3 (GTT-3)

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Abstract

Thrombus formation in a severely stenosed artery is initiated by high shear activation of platelets, with soluble platelet agonists, such as ADP and thromboxane, playing only a secondary role in the growth and stability of the thrombus. Conventional platelet function tests, however, assess only the soluble agonist-dependent pathway of platelet aggregation. As the thrombus evolves, its stability and ability to withstand dislodgement by arterial flow will determine whether complete and persistent vessel occlusion will occur. The Global Thrombosis Test (GTT), an automated point-of-care technique, simulates the formation of thrombus in whole blood under high shear flow (shear rate >12 000 s^{-1}) and measures the time for occlusive thrombus formation and spontaneous, endogenous thrombolysis/fibrinolysis. The latest GTT-3 model subjects the growing thrombus to upstream pressure, resembling that in a medium-sized artery, and provides an additional assessment of thrombus stability and fibrinolysis rate. It can be used in 3 programs, including a new "hypershear" mode, whereby repetitive cycles of pressure are applied to the growing thrombus, increasing shear rate to ~22 000 s^{-1}, such as that in patients on mechanical circulatory support. In addition to assessing the risk of arterial thrombosis, the GTT-3 could be used to assess the impact of antithrombotic medications on thrombus stability at high shear. Although current antiplatelet medications target the biochemical axis of platelet aggregation (soluble agonists) and also increase bleeding risk, novel shear-selective antiplatelet therapies may prevent thrombosis while preserving hemostasis. Future studies are needed to assess the usefulness of assessing thrombus stability on cardiovascular and pharmacological evaluation.

Keywords

arterial thrombosis, thrombotic status, thrombus stability, endogenous thrombolysis, fibrinolysis

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Introduction

The likelihood of a thrombus forming in an artery is determined at any time by the interaction of blood cells (mainly platelets and leukocytes) together with activation of coagulation, under altered hemodynamic conditions. Although arterial thrombosis is thought to occur most often in response to endothelial disruption, the finding that a microscopic, typical platelet-rich thrombus can be formed in a pierced Teflon arteriovenous shunt indicates that the presence of a vessel wall, and endothelial damage with exposure of subendothelial tissues or collagen are nonessential parts of a pathologically

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relevant in vitro hemostasis/thrombosis test.[1] Click or tap here to enter text.

At shear rates up to 10 000 s^{-1}, initial adhesion to a reactive surface and subsequent aggregation is the generally accepted mechanism of thrombus formation. However, at shear rates above 10 000 s^{-1}, activation-independent platelet aggregation, mediated by soluble von Willebrand factor (vWF), facilitates adhesion, and precedes stable aggregation.[2–4] Platelet–platelet interaction, leading to aggregation, can be induced by various soluble platelet agonists and by altered flow conditions.[4] There is strong evidence that activation of platelets by high shear stress is the first step in arterial thrombus formation in a severely stenotic artery, with soluble platelet agonists playing only a secondary role in the growth and stability of the evolving thrombus.[5]

The Global Thrombosis Test (GTT, Thromboquest Ltd) is a point-of-care test, which utilizes nonanticoagulated (native) blood, in which occlusive thrombus formation is induced solely by high, pathologically relevant, shear stress and which also allows the assessment of endogenous thrombolytic activity by detecting the onset of thrombolysis.[6]

A potential limitation of earlier models (GTT, GTT-2) is that blood flowed under atmospheric pressure under the influence of gravity, and as such, thrombi grew steadily until they occluded the small lumen. This is strikingly different from the thrombotic occlusion of an artery in vivo, in which high flow rates and arterial pressure lead to unsteady or a "staccato" dynamic thrombus growth, where the gradual growth of the thrombus is interrupted by a series of embolizations. The combination of the rate of the thrombus growth and the frequency of embolizations until occlusion occurs can be considered to reflect thrombus stability.

The present paper describes the GTT-3, the latest model, which now allows the assessment of thrombus stability both during thrombus formation, and the subsequent susceptibility to and rate of endogenous thrombolysis.

GTT-3: General Description

The principle of the GTT has previously been described in detail[6] and is shown in Figure 1. Briefly, native blood is introduced into and flows through a plastic tube in which 2 ceramic ball bearings, in series, obstruct the conical part of the tube. There are 3 narrow gaps between the ball bearings and the inner surface of the plastic tube. As blood flows through the gaps adjacent to the upper ball bearing, the resulting high initial shear rates (~12 000 s^{-1}) in this region cause platelet activation. Just downstream, in the space between the ball bearings, due to turbulent flow and low shear, the activated platelets aggregate, thrombin is generated, resulting in the formation of platelet microaggregates cross-linked by fibrin. When these microthrombi reach the gaps adjacent to the lower ball bearing, they gradually occlude these gaps, reducing the downstream flow rate and finally arresting flow. The instrument measures the time (delta, Δ) between consecutive blood drops, which increases gradually as flow slows down and at an arbitrary point (default $\Delta \geq 10$ s), the endpoint of the measurement is displayed (time of initial occlusive thrombus formation [occlusion time, OT]). The restart of blood flow following complete occlusion is due to endogenous (spontaneous) thrombolysis (lysis time, LT). If lysis does not occur until 6000 s following OT (LT cutoff time), "no lysis" is recorded.

The 4-channel instrument allows the testing of 4 patients or samples independently. The test is automated from start to finish. Data obtained during the test are simultaneously displayed and saved in a memory card, which can be viewed using the dedicated GTT-Draw software.

GTT-3: Functional Description

A typical graph drawn by the software, constructed from the recorded time intervals between consecutive blood drops (Δ) is shown in Figure 2. As soon as the blood sample is introduced into the GTT tube, blood flows under gravity until OT is reached, that is, when the interval between consecutive blood drops (Δ) exceeds 10 s. At this point, the pressure pump automatically switches on, to exert pressure on the thrombus, such that blood now flows under pressure for a preset period (default: 300 s). The total number of blood drops detected while pressure is exerted, is inversely related to the thrombus growth rate and stability, that is, the greater the number of drops, the less stable the thrombus. After the arrest of flow due to complete occlusion, the program waits for 300 s, referred to as the thrombus stabilization period (under atmospheric pressure), and then the first subsequent blood drop sensed is taken as the onset of spontaneous thrombolysis (restart of flow) and displayed as the LT. Immediately after LT is recorded, the air-pump switches on again and blood is again subjected to pressure for 600 s. The total number of blood drops during

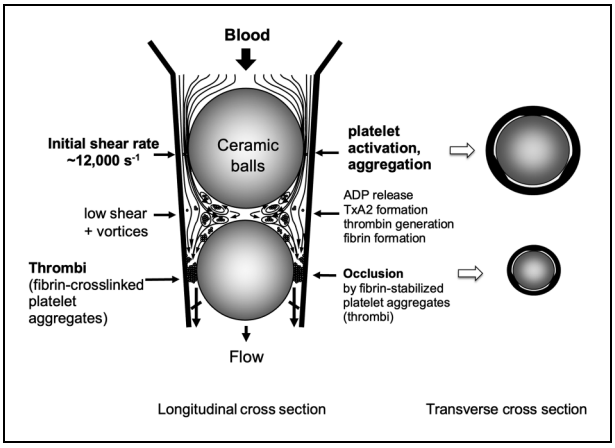

Figure 1. Schematic showing the principle of thrombus formation in the global thrombosis test. As blood flows through narrow gaps adjacent to the higher ball bearing, high shear forces in this zone cause shear-induced platelet aggregation. Downstream, in the area between the 2 ball bearings, at low shear, activated platelets begin to aggregate. These travel downstream to eventually occlude the narrow gaps adjacent to the smaller ball bearing, by occlusive thrombus.

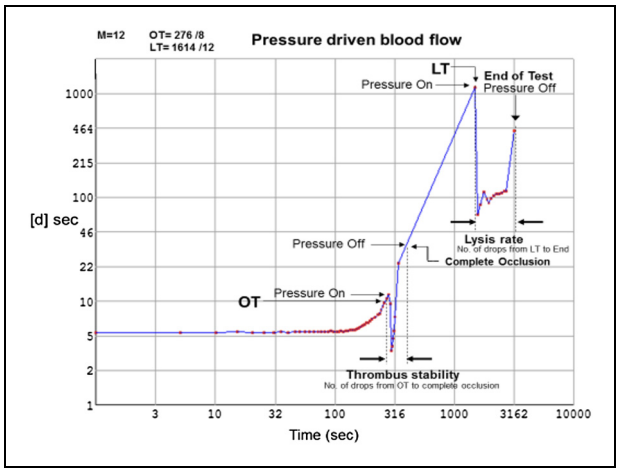

Figure 2. A typical graph showing the time intervals when blood flow is driven by pressure. From the start until the onset of occlusion, blood flows under gravity. During thrombus growth from initial to complete occlusion, pressure is applied. Stabilization of occlusion is under atmospheric pressure, but pressure is applied immediately after the onset of spontaneous thrombolysis, and the rate of lysis is determined under pressure.

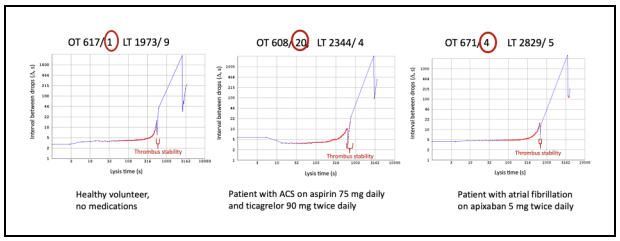

Figure 3. Examples of GTT-3 curves showing the variable effects of antithrombotic medications on thrombus stability. The red circles highlight the number of "drops" seen after initial occlusion (OT). The number of drops is inversely proportionate to the thrombus stability. The red brackets show this in a different way, on the curve, and the greater the size of the brackets (inserted here for illustration) the more unstable the thrombus.

the above pressure period is inversely related to the rate of lysis, that is, the more stable the thrombus, the fewer the number of drops. At the end of the second period of applied pressure, the measurement ends. Figure 3 shows the instrument display at the end of the measurement.

Using the GTT-3 in Different Modes

In contrast to earlier models, the GTT-3 can be used in 3 different modes, selected from the device menu: (1) GTT-3, (2) GTT-2 Basic Clinical, and (3) Hypershear modes. The user can select the mode best suited to the research question or clinical need. Since the GTT-3 mode allows the assessment of thrombus stability, this may be preferable to the GTT-Basic Clinical mode in individuals taking antiplatelet or anticoagulant medication, to assess the impact of treatment on thrombus stability. The Hypershear mode could be used to assess the effectiveness of antithrombotic medications at very high shear rates, such as those in extremely stenotic arteries, and in patients on artificial circuits including patients on mechanical circulatory support.

Optional "Hypershear" Mode

The "Hypershear mode" is a new feature of GTT-3 that was not available in earlier models. The principle is that this allows the assessment of thrombus formation and lysis under super-high shear conditions.

In the GTT-2, blood flows under the influence of gravity at a shear rate of $\approx$12 000 s^{-1} (corresponding shear stress 480 dynes/cm^2). Despite this high shear rate, which is above the conventional threshold required for vWF-induced platelet activation (10 000 s^{-1}), the rate of platelet activation in the GTT-2 is slow, as evidenced by the relatively long time from the start of the measurement until the measurable occlusion is detected. The likely reason for such a long "lag time" is that at a shear rate above 10 000 s^{-1}, platelet activation-dependent aggregation takes time until the initially unstable aggregates become stabilized, thrombin is generated, and the fibrin-stabilized thrombi cause lasting occlusion. However, when shear rate exceeds >20 000 s^{-1} (activation-independent platelet aggregation), stable aggregates are formed almost instantaneously, without the need for prior platelet activation and the subsequent release of ADP. At such very high shear rates, the antithrombotic effect of commonly used antiplatelet drugs is greatly diminished.

In the new "Hypershear mode," repetitive cycles of pressure are applied to the flowing blood to increase the flow rate and the shear rate to $\approx$22 000 s^{-1} until OT is reached (Figure 4). Each cycle consists of 5 s of pressure-driven flow at shear rate of $\approx$22 000 s^{-1}, followed by 15 s of flow under gravity at shear rate of $\approx$12 000 s^{-1}. Applying pressure in cycles greatly accelerates the rate of occlusion, reducing the OT by 30% to 50% compared to flow under gravity.

Examples of GTT-3 Measurements Showing Variable Thrombus Stability

In Figure 3, we show examples of GTT-3 measurements in individuals on no antithrombotic medications, dual antiplatelet medication, and anticoagulation. In addition to differences in OT and LT, one can also see that antithrombotic medications can significantly influence the degree of thrombus "stability," with anticoagulation and antiplatelet agents significantly destabilizing the thrombus.

Discussion

Novelty

The GTT-3, in addition to measuring the time taken to form and lyse a platelet-rich thrombus under arterial flow conditions,

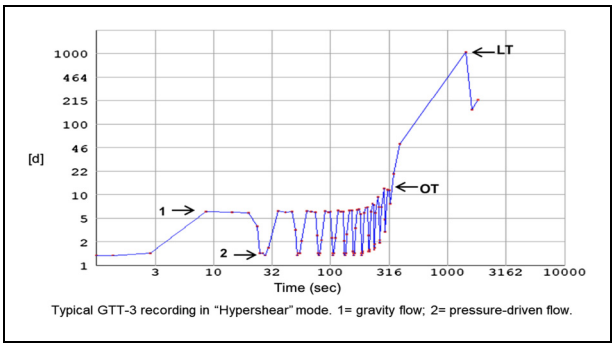

Figure 4. Typical recording in "hypershear" mode. → Gravity flow. 2. → Flow under pressure. An identical sample from the same patient tested in parallel under gravity flow showed occlusion time (OT) 531 s and lysis time (LT) 1608 s.

additionally allows the assessment of thrombus stability and the rate of spontaneous thrombolysis.

Until now, the "strength" or the stability of a growing thrombus, could be only assessed in flow chamber techniques, such as the Badimon chamber,[7] which are complex laboratory set-ups that cannot be used for routine clinical assessment, and require large volumes of blood and significant skill to set up and use.

Viscoelastic techniques, such as the TEG or the ROTEM, while displaying a graph of the rate of lysis, assess the lysis of clot formed under static or low shear conditions, most akin to venous thrombosis, and results are therefore not applicable to arterial thrombosis.

Finally, the resistance to lysis has also been assessed using the addition of external fibrinolytics, mainly to plasma clots. This has major limitations in applicability to arterial thrombosis. Firstly, the plasma clot, comprising exclusively of fibrin fibers, without cellular constituents, in particular lacking platelets, does not resemble an arterial thrombus.[8] Secondly, the rate of plasma clot lysis in response to an externally added fibrinolytic agent, such as tissue-plasminogen activator, does not reflect the effectiveness of the endogenous (spontaneous) thrombolytic function in blood.[8]

The latest refinement of this instrument, therefore, yields additional measurements that could provide additional insight into the overall thrombotic status, which appears to be highly relevant to arterial thrombosis.

Although there have been a number of published studies using the GTT-2,[8–17] there are no published studies specifically using the GTT3 in hypershear mode or reporting on the rate of fibrinolysis. Previous studies from our group have shown that endogenous fibrinolysis time measured with the GTT correlated with fibrinogen level ($r = 0.300$, $P = .001$) and hs-CRP ($r = 0.236$, $P = .011$), but was not related to other markers of coagulation or inflammation.[8] In patients with STEMI, impaired endogenous fibrinolysis was related to low albumin, raised fibrinogen, raised high-sensitivity C-reactive protein.[9] Future studies are needed to assess the usefulness of this technique in assessing the impact of various medications on the rate of endogenous fibrinolysis.

Potential Clinical Relevance

To be pathologically relevant, an in vitro thrombosis test should imitate in vivo conditions as closely as possible. Thrombus formation in a severely stenosed artery is initiated by high shear activation of platelets and disturbed flow conditions.[5,18] Thrombus growth is a dynamic process since platelet aggregation does not simultaneously proceed hand-in-hand with thrombin generation and fibrin stabilization of the loose platelet aggregate. The growth of an arterial thrombus is not a steady process but is frequently interrupted by dislodgement or embolization of small parts or the whole thrombus into the downstream circulation. As the growing thrombus gradually narrows the vessel lumen, shear stress exponentially increases. An average 10% decrease in the vessel diameter at the level of the stenosis can cause a significant increase (40%-90%) in shear stress.[19] The maximum shear rate in a severe short stenosis can exceed 250 000 s^{-1}.[20] In order to be able to resist such extreme forces, the occlusive thrombus must be stable and firmly attached to the vessel wall. A review of the mechanisms which confer stability on an evolving arterial thrombus emphasized the significance of thrombus stability in determining the clinical consequences of arterial thrombosis, namely the development or abortion of myocardial infarction or stroke.[21] Thus, the information provided on thrombus stability could, in theory, add significantly to the overall thrombotic profile. The relative importance of information on thrombus stability, over and above that provided by the OT and LT, remains to be established.

Potential Future Applications

Aside from monitoring the thrombotic status of individuals, the additional feature of assessing thrombus stability may be useful in the development and assessment of current, and more importantly, novel antithrombotic drugs which specifically target thrombus stability, without majorly affecting hemostasis.[22] Currently available antithrombotic medications to target arterial thrombosis are significantly limited by bleeding side-effects.

As discussed earlier, arterial thrombus formation occurs via 2 distinct pathways of platelet aggregation: soluble agonist-dependent (biochemical) and rheology-dependent (biomechanical) pathways. However, currently available antithrombotic medications target the biochemical axis of platelet aggregation without considering the biomechanical aspect.

The shear-dependent unfolding of vWF is crucial for platelet aggregation and pathological thrombus formation at high shear rates. Therefore, targeting vWF under the pathologically high shear rates that are uniquely present in arteries at the sites of thrombus formation might allow differentiation between thrombosis and hemostasis and enable the development of shear-selective antiplatelet therapies, to minimize thrombosis without affecting hemostasis.[23] Novel approaches of preventing

occlusive thrombi by exploiting pathological shear conditions have been explored.[23] Inhibition of the shear-driven interaction of vWF with glycoprotein (GP) Ibα has been achieved in preclinical models using monoclonal antibodies or aptamers.[23–25] Another possibility is the use of shear-sensitive vehicles such as nanoparticles or liposomes, which specifically target occlusive thrombi at abnormally high shear rates.[23,24] The recent development of a monoclonal antibody against a shear gradient-specific conformation of vWF that inhibits platelet aggregation exclusively at sites of shear rate gradients could inhibit local thrombus formation in highly stenotic vessels, while maintaining normal hemostasis.[26] Recently, studies using video microscopy have shown an important role for GPVI in maintaining thrombus stability and indicate that targeting GPVI may be an attractive way to promote platelet disaggregation in a shear-dependent manner.[27]

Conclusion

The GTT-3 simulates pathological thrombus formation under conditions of high shear, with additional information provided on thrombus stability and the rate of thrombolysis. This additional information may provide a more comprehensive overview of the overall thrombotic status. Future large studies are needed to fully explore the clinical usefulness of measuring thrombus stability and the rate of lysis.

Declaration of Conflicting Interests

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References

1. Johnson SA, Guest MM. *Dynamics of Thrombus Formation and Dissolution*. Philadelphia:Lippincott; 1969, pp. 64–71
2. Ruggeri ZM. The role of von Willebrand factor in thrombus formation. *Thromb Res*. 2007;120(Suppl 1):S5-S9.
3. Ruggeri ZM, Orje JN, Habermann R, et al. Activation-independent platelet adhesion and aggregation under elevated shear stress. *Blood*. 2006;108(6):1903-1910.
4. Jackson SP, Nesbitt WS, Westein E. Dynamics of platelet thrombus formation. *J Thromb Haemost*. 2009;7(Suppl 1):17-20.
5. Nesbitt WS, Westein E, Tovar-Lopez FJ, et al. A shear gradient-dependent platelet aggregation mechanism drives thrombus formation. *Nat Med*. 2009;15(6):665-673.
6. Sharma S, Farrington K, Kozarski R, et al. Impaired thrombolysis: a novel cardiovascular risk factor in end-stage renal disease. *Eur Heart J*. 2013;34(5):354-363.
7. Zafar MU, Santos-Gallego CG, Badimon L, et al. Badimon perfusion chamber: an ex vivo model of thrombosis. *Methods Mol Biol*. 2018;1816:161-171. doi: 10.1007/978-1-4939-8597-5_12
8. Kanji R, Gue YX, Farag MF, et al. Determinants of endogenous fibrinolysis in whole blood under high shear in patients with myocardial infarction. *JACC Basic Transl Sci*. 2022;7(11):1069-1082.
9. Farag M, Spinthakis N, Gue YX, et al. Impaired endogenous fibrinolysis in ST-segment elevation myocardial infarction patients undergoing primary percutaneous coronary intervention is a predictor of recurrent cardiovascular events: the RISK PPCI study. *Eur Heart J*. 2019;40(3):295-305.
10. Yamashita T, Sato A, Ikarugi H, et al. Significantly reduced spontaneous thrombolytic activity in older men: a possible explanation for the gender differences in risk of acute coronary syndromes. *Thromb Res*. 2005;116(2):127-131.
11. Gorog DA, Yamamoto J, Saraf S, et al. First direct comparison of platelet reactivity and thrombolytic status between Japanese and Western volunteers: possible relationship to the "Japanese paradox". *Int J Cardiol*. 2011;152(1):43-48.
12. Suehiro A, Wakabayashi I, Uchida K, et al. Impaired spontaneous thrombolytic activity measured by global thrombosis test in males with metabolic syndrome. *Thromb Res*. 2012;129(4):499-501.
13. Suehiro A, Wakabayashi I, Yamashita T, et al. Attenuation of spontaneous thrombolytic activity measured by the global thrombosis test in male habitual smokers. *J Thromb Thrombolysis*. 2014;37(4):414-418.
14. Farag M, Niespialowska-Steuden M, Okafor O, et al. Relative effects of different non-vitamin K antagonist oral anticoagulants on global thrombotic status in atrial fibrillation. *Platelets*. 2016;27(7):687-693.
15. Niespialowska-Steuden M, Markides V, Farag M, et al. Catheter ablation for AF improves global thrombotic profile and enhances fibrinolysis. *J Thromb Thrombolysis*. 2017;44(4):413-426.
16. Spinthakis N, Gue Y, Farag M, et al. Apixaban enhances endogenous fibrinolysis in patients with atrial fibrillation. *Europace*. 2019;21(9):1297-1306.
17. Kanji R, Gue YX, Memtsas V, et al. Point-of-care biomarkers of thrombotic status predict spontaneous reperfusion in ST-segment elevation MI and clinical outcomes. *J Am Coll Cardiol*. 2023 May 16;81(19):1918–1932.
18. Jackson SP. Arterial thrombosis–insidious, unpredictable and deadly. *Nat Med*. 2011;17(11):1423-1436.
19. Maalej N, Folts JD. Increased shear stress overcomes the antithrombotic platelet inhibitory effect of aspirin in stenosed dog coronary arteries. *Circulation*. 1996;93(6):1201-1205.
20. Bark DL Jr, Ku DN. Wall shear over high degree stenoses pertinent to atherothrombosis. *J Biomech*. 2010;43(15):2970-2977.

21. Gorog DA, Fayad ZA, Fuster V. Arterial thrombus stability: does it matter and can we detect it? *J Am Coll Cardiol*. 2017;70(16):2036-2047.
22. Angelillo-Scherrer A. Targeting thrombus stabilization: protection against thrombosis without bleeding? *Drug Disc Today*. 2004;1(3):279-287.
23. Rana A, Westein E, Niego B, et al. Shear-dependent platelet aggregation: mechanisms and therapeutic opportunities. *Front Cardiovasc Med*. 2019 Sep 20;6:141.
24. Chen Y, Ju LA. Biomechanical thrombosis: the dark side of force and Dawn of mechano-medicine. *Stroke Vasc Neurol*. 2020;5(2):185-197.
25. Sakai K, Someya T, Harada K, et al. Novel aptamer to von Willebrand factor A1 domain (TAGX-0004) shows total inhibition of thrombus formation superior to ARC1779 and comparable to caplacizumab. *Haematologica*. 2020;105(11):2631-2638.
26. Hoefer T, Rana A, Niego B, et al. Targeting shear gradient activated von Willebrand factor by the novel single-chain antibody A1 reduces occlusive thrombus formation in vitro. *Haematologica*. 2021;106(11):2874-2884.
27. Ahmed MU, Kaneva V, Loyau S, et al. Pharmacological blockade of glycoprotein VI promotes thrombus disaggregation in the absence of thrombin. *Arterioscler Thromb Vasc Biol*. 2020; 40(9):2127-2142.