Endogenous fibrinolysis - relevance to clinical thrombosis risk assessment

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Running title: Endogenous fibrinolysis in clinical practice

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Abstract

The development of an obstructive luminal thrombus is pathological and considered a failure of endogenous fibrinolysis. The consequences may be fatal, or result in lasting downstream organ damage. Therefore, assessment of endogenous fibrinolytic status in an individual may identify those at risk of occlusive thrombus formation and provide prognostic information. Arterial thrombi are more platelet-rich and more resistant to fibrinolysis than venous thrombi. Several recent studies using global tests of fibrinolysis in patients with acute coronary syndromes (ACS) have shown that despite dual antiplatelet therapy, patients with impaired fibrinolytic status have an increased risk of adverse cardiovascular events, compared to those with effective fibrinolytic function. Such data add significantly to the predictive value of established cardiovascular risk factors and conventional biomarkers. Most data reported have been obtained with the Global Thrombosis Test and the turbidimetric plasma clot lysis assay. A few small studies in patients with ischaemic stroke suggest a similar predictive role of fibrinolytic status assessment in these patients. Studies reporting an association between impaired fibrinolysis and future venous thrombotic events are limited, and in the form of case-control studies. Viscoelastic assays may have a role in the prediction of venous thromboembolic risk.

Assays of fibrinolytic function should be used to obtain a more accurate risk of future thrombotic events, particularly in the setting of ACS. The availability of point-of-care tests helps facilitate this and should encourage future studies to assess personalised antithrombotic treatment combinations to optimise fibrinolytic status and reduce thrombosis risk.

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Abbreviations

ACS = acute coronary syndrome

CLT = clot lysis time

DVT = deep vein thrombosis

ECL = euglobulin clot lysis

GTT = Global Thrombosis Test

LT = lysis time

MACE = major adverse cardiovascular event

MI = myocardial infarction

OT = occlusion time

PAI-1 = plasminogen activator inhibitor-1

ROTEM = rotational thromboelastometry

STEMI = ST-segment elevation myocardial infarction

TAFI = thrombin activatable fibrinolysis inhibitor

TEG = thromboelastography

t-PA = tissue plasminogen activator

VTE = venous thromboembolism

Introduction

Arterial and venous thrombotic events are important causes of mortality and morbidity worldwide,[1] and antithrombotic therapy is the cornerstone in prevention and treatment. Platelet aggregation and activation of the coagulation cascade are common to the aetiology of both arterial and venous thrombotic events. Whilst treatment for these conditions generally involves antiplatelet therapy and anticoagulation respectively, despite such pharmacotherapy, some patients experience recurrent thrombotic events.[2] Current antiplatelet therapies such as prasugrel and ticagrelor achieve potent platelet inhibition, yet the proportion of patients experiencing recurrent thrombotic events is higher than the number of individuals who exhibit suboptimal inhibition of platelet aggregation. Thrombus formation represents a complex interplay between procoagulant factors, namely those stimulating platelet activation and activation of coagulation, and fibrinolytic factors. The administration of fibrinolytic agents in the setting of ischaemic stroke and myocardial infraction can achieve clot lysis and restoration of vessel patency in a significant number of individuals.[3][4]

Impaired endogenous fibrinolysis is one mechanism that may be responsible for thrombotic events that occur despite conventional antithrombotic therapy. In this review, we discuss drivers of arterial and venous thrombotic events, and review the evidence supporting the role of impaired endogenous fibrinolysis in the aetiology of these events. We discuss ways to measure fibrinolysis, and how this can identify patients who remain at risk of future thrombotic events.

Arterial thrombosis

Arterial thrombotic events are usually initiated by platelet activation, secondary to plaque disruption, through the release of tissue factor and exposure of collagen, and/or platelet activation due to high shear at the site of a severe luminal stenosis (Figure 1).[5] Disruption of an atherosclerotic plaque due to rupture or fissure exposes platelets to subendothelial contents causing platelet activation and localised aggregation. In response to high shear, the degree of platelet activation is proportional to the severity of the applied shear forces,[6] and may be further augmented by platelet hammering,[7] whereby platelets are exposed repeatedly to high shear.

Platelet activation and adhesion to the site of injury, aided by von Willebrand Factor, allows growth of the locally-anchored platelet thrombus. The strength of adhesion of the growing thrombus to the vessel wall is an important determinant of thrombus stability and determines the risk of embolization. Local activation of coagulation results in thrombin generation, leading to the conversion of fibrinogen to fibrin, creating a fibrin mesh to bind together the growing platelet aggregate. Studies have highlighted the direct relationship between the concentration of thrombin and the fibrin fibre thickness and density.[8] *In situ* thrombin generation is critical for imparting stability to the growing thrombus, and shear-activated platelets express phosphatidylserine which facilitates thrombin generation on the platelet surface.[9]

In addition to factors that impart structural stability, the critical determinant of whether the thrombus will continue to grow and cause vessel occlusion, is the effectiveness of the endogenous fibrinolytic system.

Endogenous fibrinolysis, responsible for the breakdown of thrombus, involves many complex interactions between activators and inhibitors, ultimately leading to the enzymatic cleavage of fibrin by plasmin. Plasmin circulates as an inactive precursor, plasminogen, and upon binding to thrombus, is converted into its active form by urokinase-plasminogen activator (uPA), or tissue-plasminogen activator (t-PA), produced by the endothelium. Regulating the function of t-PA is plasminogen activator inhibitor-1 (PAI-1), which binds to the active site of t-PA and prevents the binding of plasminogen. This is also produced by the endothelium, and other tissues such as adipose. In addition to plasmin-mediated fibrinolysis, leukocytes entrapped within the thrombus contribute to the breakdown of fibrin through the release of elastase and cathepsin G.[10]

There are also many processes and proteins which inhibit fibrinolysis, such as thrombin, through the activation of thrombin activatable fibrinolysis inhibitor (TAFI). Further, activated platelets inhibit fibrinolysis through the release of stored PAI-1 from their alpha granules,[11] the expression of factor XIII on the platelet surface[12] and clot retraction. Factor XIII is responsible for crosslinking the fibrinolysis inhibitor alpha-2-antiplasmin with fibrin, thereby preventing plasmin-mediated fibrin degradation, and restricting the entry of fibrinolytic enzymes to the thrombus core by reducing pore size. Clot retraction, which reduces clot volume by 30-40%, results in enhanced resistance to lysis.[13][14] Alpha-2-antiplasmin also has an additional role in sequestering plasmin in the circulation to form a complex, and thereby preventing its entry into the thrombus. Cell mediated-effects including the release of

DNA and other proteins which form neutrophil extracellular traps (NETS), can initiate coagulation,[15] and enhance resistance to fibrinolysis.

Venous thrombosis

The pathogenesis of venous thrombosis differs from that of arterial thrombosis, and is based on Virchow's triad: stasis, hypercoagulability and vascular injury (Figure 1). Prolonged stasis of blood in small pockets adjacent to venous valves[16] results in hypoxia and increased haematocrit. This reduces the expression of antithrombotic proteins on venous valves,[17] and increases expression of p-selectin, a prothrombotic chemoattractant, which results in the delivery of tissue factor to the endothelium,[18][19] initiating coagulation. The primary event may also be endothelial activation, with consequent attachment of platelets and leukocytes and expression of tissue factor. Owing to the low flow state, the balance tips in favour of coagulation, resulting in thrombosis.

Compared to arterial thrombi, venous thrombi contain relatively more erythrocytes and fewer platelets. Since platelets impart such a significant stabilising effect to the growing arterial thrombus, this may in part explain why venous thrombi with lower platelet content are less resistant to thrombolysis, and more prone to embolise.

However, growing evidence highlights the varying fibrinolytic potential of even venous clots. Resistance to lysis is exhibited by changes in fibrin structure when bound to erythrocytes,[20] and further, during clot retraction, changes to the structure and function of erythrocytes can impair fibrinolysis.[21] This, together with other mechanisms provides variability in fibrinolytic potential and may explain the embolic risk. Clots extracted from patients who have experienced both deep vein thrombosis (DVT) and pulmonary embolism (PE) compared with DVT alone, have shown greater clot permeability and lower fibrin fibre density.[22] Such structural characteristics have been shown to improve fibrinolysis, and may therefore lead to an increased risk of fragmentation and embolization. This is supported by computational models highlighting the greater embolization risk in patients with greater clot permeability compared with those clots resistant to lysis.[23]

Assessment of endogenous fibrinolysis

Whilst the importance of endogenous fibrinolysis in determining the outcome of a growing thrombus has been appreciated for decades, the measurement of fibrinolysis has been a challenge. In contrast to the availability of an armamentarium of tests to assess platelet

function, tests of fibrinolysis have evolved more slowly, and only in the last few years has research in this field has gained significant momentum. The main challenges historically were that several inhibitors and activators of fibrinolysis could be measured, but this did not build a complete "picture" of the overall fibrinolytic status. In this section, we will review the commonly used methods (Table 1).

Assessment of level or activity of individual potentiators or inhibitors of fibrinolysis

Factorial assays

Coagulation and fibrinolysis require multiple steps, governed by activators and inhibitors, including t-PA, plasmin, PAI-1, TAFI, alpha-2-antiplasmin, thrombin, factor XIIIa and lipoprotein(a). These are produced in different locations, have differing magnitudes of effect, and further, their concentrations may be augmented locally in response to stimulus. This is difficult to predict prior to an event, and furthermore, predicting the interaction of these biomarkers and factors is difficult. Measuring the level and activity of one activator or inhibitor is unlikely therefore to yield prognostic data, and modelling these interactions relies on multiple assumptions.

This is exemplified by the assessment of t-PA. Increased levels may indicate reduced propensity to lasting thrombotic occlusion, however high t-PA levels are in fact associated with greater cardiovascular risk.[24][25] This can be explained by the fact that not all t-PA is active, and it is likely that the majority is sequestered by PAI-1. Measuring activity of free t-PA therefore, would be more accurate. Similarly, in a case control study of 600 patients with myocardial infarction (MI), high TAFI level interestingly was found to be associated with reduced risk.[26] Thus, measuring isolated levels of proteins can be misleading, and this view is supported by a review of 45 studies, with nearly 50,000 patients.[27] It highlights the lack of concordance between studies measuring inhibitors and activators of fibrinolysis to predict outcome. Therefore, it is unlikely that factorial assays will aid risk stratification in the future.

Global assessment of fibrinolysis in response to externally-added activators of coagulation and lysis

Euglobulin Clot Lysis (ECL)

This rather outdated technique has largely been replaced by novel methods. It involves the collection of citrated plasma, which is then acidified and incubated to form a precipitant,

termed the euglobulin fraction (which contains little in the way of fibrinolysis inhibitors) and to which calcium is added to initiate clotting. The time taken to lyse this clot is then measured.

Plasma Clot Lysis Time (CLT)

This test also involves the use of citrated platelet-poor plasma which is obtained by centrifugation, but unlike ECL, has no inhibitors or activators removed. Instead, clotting in the plasma in well-plates is activated by the addition of an activation mixture containing calcium and thrombin, and fibrinolysis initiated by the addition of lysis mix containing plasminogen activator. Aside from the initial mixing, lasting a few seconds, both clotting and lysis thereafter takes place in static conditions, and consequent changes in turbidity are used as surrogates for thrombus formation and lysis. Fibrinolysis is measured as the time taken for maximum turbidity to drop by 50%.

Both CLT and ECL have significant limitations in assessing fibrinolysis. They effectively exclude from assessment the cellular constituents of blood, which make a significant contribution to thrombosis and fibrinolysis. Furthermore, these tests are reliant on the external addition of thrombin to initiate clot formation and t-PA to initiate fibrinolysis (or removal of inhibitors in the case of ECL), so these do not measure endogenous processes but rather the response to external agonists or activators. Finally, the effect of flow and shear are not assessed since these are static tests.

<u>Global assessment of endogenous fibrinolysis</u>

Global Thrombosis Test

The Global Thrombosis Test (Thromboquest Ltd., London, United Kingdom) is perhaps the only technique that truly measures endogenous fibrinolysis in whole blood. The technique is point-of-care and fully automated. It utilises non-anticoagulated whole blood which is subjected to flow under high shear, which results in platelet activation and aggregation. This eventually results in arrest of blood flow due to occlusive thrombus formation. The instrument then assesses the time taken for spontaneous restart of blood flow due to endogenous fibrinolysis. The thrombus formation time (OT) and lysis time (LT) are detected using a built-in photometer. It provides a physiological assessment of the effectiveness of endogenous fibrinolysis, since no external agonists to initiate coagulation or lysis are added. Whilst highly physiological, since it also employs whole blood and high shear, the physiological relevance of the test by employing non-anticoagulated blood, comes at the price of needing to perform the test immediately on freshly drawn blood.

Viscoelastic tests

These tests include thromboelastography (TEG; Haemonetics Corporation, Illinois, USA) and rotational thromboelastometry (ROTEM; Tem Innovations GmbH, Munich, Germany). These tests assess whole blood and under static coagulation (slow rotational force applied), measure the time for clot formation and spontaneous lysis, based on changes detected in viscosity. The tests provide information on clot formation, propagation, stabilisation, and dissolution, and therefore provide a global assessment of haemostasis and clot lysis. Although similar in principle, there are differences between the TEG and ROTEM. With TEG, either whole or citrated blood can be used (citrated blood can be collected and stored for up to two hours at room temperature and must be re-calcified prior to analysis). The blood is placed within a disposable cup which undergoes a constant rotational force, mimicking a low flow state, similar to that in the venous system. A pin is suspended in the blood, and as clot forms and lyses, varying forces are exerted on the pin, which are measured. Specifically, the instrument records the time taken to form an initial clot, the speed thereafter to generate the final clot, clot strength and percentage clot lysis at 30 and 60 minutes. A global assessment clot formation and lysis is therefore obtained. ROTEM is a later derivative of TEG, and is fully automated, with the major difference being that the pin, instead of the cup undergoes a constant rotational force.

The main limitations of these techniques are that arterial flow conditions and platelet activation are not replicated in these tests. In fact, the low shear conditions in these tests

assesses coagulation and clot lysis that better reflect venous thrombosis. Results can take approximately 2 hours, which can be hastened with the addition of an activator, namely tissue factor in r-TEG, but this renders the test less physiological, assessing the response to external agents rather than native, endogenous fibrinolysis.

Assessment of fibrinolysis in relation to the prediction of arterial thrombotic events

The effectiveness of fibrinolysis may determine an individual's thrombotic risk and the effectiveness of pharmacotherapy given to treat or prevent arterial thrombosis (Figure 2). Fibrin structure has been associated with several thrombotic conditions. Patients with severe coronary artery disease have been shown to produce thrombi containing dense fibrin networks with reduced permeability. In patients with ST-segment elevation myocardial infarction (STEMI), there is growing data to support histological differences in clot structure, specifically in relation to fibrin content, which relates to resistance to thrombolysis.[28][29][30]
Therefore, measuring fibrinolytic potential may help to identify individuals at increased risk of future thrombotic events, and amongst those with acute thrombosis, identify those at risk of treatment failure due to persistent arterial occlusion of the microcirculation, and future recurrent events.

Myocardial infarction

Patients presenting with STEMI who achieve spontaneous reperfusion, likely due to effective endogenous fibrinolysis, have a more favourable outcome, including lower mortality, recurrent infarction and heart failure.[31][32] However, in most reported case series, spontaneous reperfusion occurred in only 15-22% of patients with STEMI.[31][32]
Global tests show promise in identifying impaired fibrinolysis in the setting of acute coronary syndromes (ACS) (Figure 1). One of the largest studies assessing the prognostic impact of impaired fibrinolysis in patients with ACS was a sub-study of the PLATelet inhibition and patient Outcomes (PLATO) study.[33] In 4,354 patients hospitalised for ACS, plasma CLT and fibrin clot density were assessed by adding both lysis and activation mix to platelet-poor plasma, to stimulate clotting and then fibrinolysis. At peak clot formation, maximum turbidity was recorded and used as a surrogate for fibrin clot density. Thereafter, the time taken for the turbidity to drop by 50%, termed the lysis time, was recorded as fibrinolysis potential. The CLT correlated with cardiovascular death and MI at 1 year after adjusting for

cardiovascular risk factors (HR 1.17, 95% confidence interval [CI] 1.05-1.31; p<0.01) and correlated with cardiovascular death after adjustment for known prognostic biomarkers (HR 1.2, 95% CI 1.01-1.42; p=0.042). Maximum turbidity was associated with increased cardiovascular death (HR 1.24, 95% CI 1.03-1.50, p=0.024), but not after adjustment for known prognostic biomarkers.[33] In a sub-group of 974 of patients with diabetes, increased CLT remained associated with increased cardiovascular death and MI including after adjustment for multiple biomarkers.[34]

Studies using the Global Thrombosis Test have shown even better risk prediction with the assessment of endogenous fibrinolysis. In a study of 300 patients with ACS, impaired endogenous fibrinolysis, defined as a lysis time >3000s, was associated with an increase in 1 year major adverse cardiovascular events (MACE) (HR 2.52, 95% CI 1.34–4.71, p=0.004) and cardiovascular death (HR 4.2, 95% CI 1.13–15.62, p=0.033).[35] In another more recent study of 496 patients with STEMI, impaired endogenous fibrinolysis, assessed immediately on arrival to the catheterisation laboratory, was highly predictive of MACE (HR 9.1, 95% CI 5.29–15.75; p<0.001), cardiovascular death (HR 18.5, 95% CI 7.69–44.31; p<0.001) and myocardial infarction (HR 6.2, 95% CI 2.64–14.73; p<0.001) at 1 year.[36] The association remained significant after adjustment for baseline cardiovascular risk factors, and in fact, the addition of endogenous fibrinolysis improved a risk model which included known cardiovascular risk factors by over 50%.

Whilst direct comparison of the results from the Global Thrombosis Test and plasma clot lysis studies cannot be made, the difference in hazard ratios is thought provoking, and raises the hypothesis that the Global Thrombosis Test may be superior at identifying impaired endogenous fibrinolysis, perhaps because it takes account of other non-fibrin related determinants including platelets, neutrophils and thrombin generation. Through identification of high risk patients, global tests of fibrinolysis may allow personalised and targeted treatment to reduce future cardiovascular risk, for example by using a factor Xa inhibitor, which has been shown to reduce the resistance to fibrinolysis in whole blood clots,[37] and has translated clinically into a reduction in events in patients with atherosclerotic cardiovascular disease.[38][39]

Ischaemic stroke

There is growing evidence of differences in clot structure amongst patients with ischaemic stroke (Figure 1). Studies have identified an association between clot composition and

embolectomy procedure time, with fibrin rich clots being difficult to retrieve and at risk of embolisation.[40]

Factorial assays in patients with stroke have been small and lacking in power but do appear to highlight an association with outcome. In 109 patients with ischaemic stroke, higher admission TAFI activity was associated with a more severe National Institutes of Health Stroke Scale and disability, in both patients receiving and not receiving thrombolysis.[41] In another study of 43 patients with ischaemic stroke treated with thrombolysis, a significantly higher admission PAI-1 antigen level was found in the cohort of patients with failed culprit cerebral artery recanalization on angiography.[42] Interestingly, no difference in TAFI antigen level was found between the recanalized and non-recanalized cohorts.

Studies assessing CLT in patients with ischaemic stroke are still limited by size. In a study of 74 patients with ischaemic stroke, short CLT on admission was predictive of good outcome (modified Rankin Scale of 0-1) at 3 months.[43] In 335 patients, of whom 103 had had an ischaemic stroke, patients had a significantly longer CLT than controls.[44] Another study which assessed 236 patients with atrial fibrillation treated with a vitamin K antagonist, showed that plasma CLT in addition to the CHA_2DS_2-VASc score was predictive of future events over a median follow-up of 4.3 years.[45] A CHA_2DS_2-VASc score > 3 alone was associated with a HR of 2.49 for cerebrovascular ischaemic events, but when combined with a CLT >115 minutes, this portended a 10.6-fold increase in risk of CVA or TIA.

The Global Thrombosis Test was used to assess 185 patients two weeks after an acute cerebrovascular event, who were compared with 195 healthy volunteers.[46] Despite antiplatelet therapy, the endogenous fibrinolytic function of patients was significantly impaired compared to healthy volunteers, as evidenced by a longer lysis time (3159±1549s vs. 2231±1223s, $p<0.0001$). Interestingly, prior to antiplatelet therapy, the LT was prolonged even further in patients. This indicates the need for further prospective studies utilising the Global Thrombosis Test and CLT to assess endogenous fibrinolytic function in patients with ischaemic stroke, to confirm association with outcome and potentially individualise treatment. Efforts should certainly be directed towards these, rather than viscoelastic tests. A small study of 143 subjects using the ROTEM found no difference in coagulation and fibrinolysis parameters between patients and controls or between fibrinolytic status and stroke severity.[47] In a study of 171 patients with ischaemic stroke, a blood draw for

assessment by TEG was taken before and 10 minutes after administration of t-PA.[48] The TEG was unable to detect which patients clinically responded and which did not to t-PA.

Assessment of fibrinolysis in relation to the prediction of venous thrombosis

The largest studies assessing the role of fibrinolysis measurement in prediction of venous thrombotic events employed plasma clot lysis assays. Three case control studies including 400 patients with DVT,[49] 579[50] and 2090[51] patients with DVT and pulmonary embolism, showed an approximate two-fold increase in the prevalence of venous thrombotic events in patients with a prolonged CLT (Figure 1). Furthermore, a synergistic effect was seen between the detection of hypofibrinolysis and other risk factors for thrombosis, which were additive in the risk of venous thromboembolism (VTE). Patients with prolonged CLT and taking the oral contraceptive pill had a 20-fold increased risk of venous thrombosis compared with women not on the contraceptive pill and without prolonged CLT.[51] However, whilst potentially predictive of VTE in high-risk patients, the role of CLT in predicting future events and guiding treatment is not so clear. One study of 704 patients experiencing their first unprovoked VTE, showed that recurrence after termination of anticoagulation occurred in 19.2% of patients over a mean follow-up of 46 months,[52] but baseline CLT was not predictive of recurrence, but when CLT was split into quartiles, it was predictive only in women. Fibrinolysis function can also risk assess patients for subsequent complications after DVT, since patients with residual vein obstruction appear to have prolonged CLT compared to controls,[53] and patients developing chronic thromboembolic disease also demonstrate impaired lysis.[54]

The use of viscoelastic methods (TEG/ROTEM) to determine fibrinolytic function may yield superior risk stratification, as venous clots are erythrocyte-rich, and formed under conditions of low shear. These tests better mimic the venous physiological environment, compared with CLT. However, studies employing these methods to specifically assess fibrinolysis function in relation to the risk of VTE are limited. A hypercoagulable state was shown to be moderately predictive of thromboembolic events in a recent systematic review including data of some 10,000 patients utilising both TEG and ROTEM (sROC curve = 0.70, 95% CI 0.65-0.75), with OR 3.1 (95% CI 2.0-4.8).[55] The majority of studies included in the analysis used maximum clot strength to define a hypercoagulable state, with the minority using R-time, K-time and α-angle either individually, or in combination as the coagulation index.

Recently, interest has grown in the assessment of endogenous fibrinolysis to guide treatment and predict mortality in trauma patients, who are at risk of both thrombosis and bleeding. In a study of 2,540 trauma patients, in-hospital mortality rate was 21%, and greatest in those with hyperfibrinolysis as detected with TEG.[56] This was followed by fibrinolytic shutdown. After adjustment, the risk of mortality remained increased for both groups (hyperfibinolysis OR 3.3, 95% CI 2.4-4.6 p<0.0001, fibrinolytic shutdown OR 1.6, 95% CI 1.3-2.1 p=0.0003). In another study of 303 trauma patients, thromboelastometry identified the highest risk patients with severe hyperfibrinolysis.[57]

COVID-19 and fibrinolysis

Patients with COVID-19 are at risk of VTE and *in situ* pulmonary thrombosis. A few small studies implicate impaired fibrinolysis in the pathophysiology of COVID-19 infection. In a retrospective study of 21 critically-ill patients, eleven patients were shown to have hypofibrinolysis using ROTEM, of whom 8 had confirmed VTE.[58] Another study of 23 patients using ROTEM, showed that 30 minute fibrinolysis was significantly reduced in patients with compared to patients without VTE (82±26% vs. 37±35%).[59] Among 44 patients, complete fibrinolysis shutdown, defined as absence of fibrinolytic activity 30 minutes after the formation of an *in vitro* clot, was found in 57% of patients and was the best predictor of VTE (area-under-curve 0.742, p=0.021).[60]

Conclusion

Measurement of endogenous fibrinolytic potential with the point-of-care GTT, or laboratory assay of CLT, in patients with ACS provides important additional prognostic information, over and above standard cardiovascular risk factor and conventional biomarker modelling. Small studies in patients with ischaemic stroke also show that endogenous fibrinolysis may also predict outcome and response to treatment. Assessment of clot lysis with ROTEM or TEG shows moderate ability to identify patients at increased risk of VTE.

Future directions

Assessment of fibrinolysis could be used to risk stratify individuals for future arterial and venous thrombotic events. The combination of the assessment of platelet reactivity, together

with assessment of fibrinolysis, may be useful and previous studies have shown a weak negative relationship between the degree of platelet reactivity and the effectiveness of endogenous fibrinolysis.[35][36] Since impaired endogenous fibrinolysis is predictive of adverse outcome in ACS and stroke, studies are required to investigate the effects of pharmacotherapies to improve endogenous fibrinolysis.

References

1. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet (London, England) [Internet]. 2012 Dec 15 [cited 2020 Sep 18];380(9859):2095–128. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23245604
2. Spertus J, Bhandary D, Fonseca E, Bhalla N, Manzi MA, Hoetzer G, et al. Contemporary incidence of recurrent cardiovascular events 1 to 3 yeasr after myocardial infarction: longitudinal US analysis from NCDR registries linked with all-payer claims database. J Am Coll Cardiol [Internet]. 2018 Mar 10 [cited 2020 Sep 18];71(11):A24. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0735109718305655
3. Bagoly Z, Szegedi I, Kálmándi R, Tóth NK, Csiba L. Markers of Coagulation and Fibrinolysis Predicting the Outcome of Acute Ischemic Stroke Thrombolysis Treatment: A Review of the Literature. Front Neurol [Internet]. 2019 Jun 21 [cited 2020 Sep 13];10:513. Available from: https://www.frontiersin.org/article/10.3389/fneur.2019.00513/full
4. Cannon CP, Gibson CM, McCabe CH, Adgey AA, Schweiger MJ, Sequeira RF, et al. TNK-tissue plasminogen activator compared with front-loaded alteplase in acute myocardial infarction: results of the TIMI 10B trial. Thrombolysis in Myocardial Infarction (TIMI) 10B Investigators. Circulation [Internet]. 1998 [cited 2020 Oct 4];98(25):2805–14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9860780
5. Massai D, Soloperto G, Gallo D, Xu XY, Morbiducci U. Shear-induced platelet activation and its relationship with blood flow topology in a numerical model of stenosed carotid bifurcation. Eur J Mech - B/Fluids [Internet]. 2012 Sep 1 [cited 2020 Sep 12];35:92–101. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0997754612000453
6. Holme PA, Ørvim U, Hamers MJAG, Solum NO, Brosstad FR, Barstad RM, et al. Shear-Induced Platelet Activation and Platelet Microparticle Formation at Blood Flow Conditions as in Arteries With a Severe Stenosis. Arterioscler Thromb Vasc Biol [Internet]. 1997 Apr [cited 2020 Sep 18];17(4):646–53. Available from: https://www.ahajournals.org/doi/10.1161/01.ATV.17.4.646
7. Sheriff J, Tran PL, Hutchinson M, DeCook T, Slepian MJ, Bluestein D, et al. The platelet hammer: In vitro platelet activation under repetitive hypershear. In: 2015 37th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC) [Internet]. IEEE; 2015 [cited 2020 Sep 13]. p. 262–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26736250
8. Wolberg AS. Thrombin generation and fibrin clot structure. [cited 2020 Sep 18]; Available from: www.elsevierhealth.com/journals/blre
9. Reddy EC, Rand ML. Procoagulant Phosphatidylserine-Exposing Platelets in vitro and in vivo. Front Cardiovasc Med [Internet]. 2020 Mar 3 [cited 2020 Sep 18];7:15. Available from: https://www.frontiersin.org/article/10.3389/fcvm.2020.00015/full
10. Bach-Gansmo ET, Halvorsen S, Godal HC, Skjønsberg OH. D-dimers are degraded by human neutrophil elastase. Thromb Res [Internet]. 1996 Apr 15 [cited 2020 Sep 13];82(2):177–86. Available from: https://www.sciencedirect.com/science/article/abs/pii/0049384896000643
11. Erickson LA, Ginsberg MH, Loskutoff DJ. Detection and partial characterization of an inhibitor of plasminogen activator in human platelets. J Clin Invest [Internet]. 1984

Oct [cited 2020 Oct 6];74(4):1465–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/6434594
12. Mitchell JL, Lionikiene AS, Fraser SR, Whyte CS, Booth NA, Mutch NJ. Functional factor XIII-A is exposed on the stimulated platelet surface. Blood [Internet]. 2014 Dec 18 [cited 2020 Sep 13];124(26):3982–90. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25331118
13. Gorog DA, Fayad ZA, Fuster V. Arterial Thrombus Stability: Does It Matter and Can We Detect It? J Am Coll Cardiol [Internet]. 2017 Oct 17 [cited 2020 Sep 13];70(16):2036–47. Available from: https://www.sciencedirect.com/science/article/pii/S0735109717395372?via%3Dihub
14. Shi X, Yang J, Huang J, Long Z, Ruan Z, Xiao B, et al. Effects of different shear rates on the attachment and detachment of platelet thrombi. Mol Med Rep [Internet]. 2016 Mar [cited 2020 Sep 13];13(3):2447–56. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26847168
15. Martinod K, Wagner DD. Thrombosis: tangled up in NETs. Blood [Internet]. 2014 May 1 [cited 2020 Sep 13];123(18):2768–76. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24366358
16. Nicolaides AN, Kakkar V V, Field ES, Renney JT. The origin of deep vein thrombosis: a venographic study. Br J Radiol [Internet]. 1971 Sep [cited 2020 Sep 13];44(525):653–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/5569959
17. Brooks EG, Trotman W, Wadsworth MP, Taatjes DJ, Evans MF, Ittleman FP, et al. Valves of the deep venous system: an overlooked risk factor. Blood [Internet]. 2009 Aug 6 [cited 2020 Sep 13];114(6):1276–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19436051
18. Myers DD, Hawley AE, Farris DM, Wrobleski SK, Thanaporn P, Schaub RG, et al. P-selectin and leukocyte microparticles are associated with venous thrombogenesis. J Vasc Surg [Internet]. 2003 Nov [cited 2020 Sep 13];38(5):1075–89. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14603220
19. Closse C, Seigneur M, Renard M, Pruvost A, Dumain P, Belloc F, et al. Influence of hypoxia and hypoxia-reoxygenation on endothelial P-selectin expression. Haemostasis [Internet]. 1996 Oct [cited 2020 Sep 13];26 Suppl 4:177–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8979122
20. Wohner N, Sótonyi P, Machovich R, Szabó L, Tenekedjiev K, Silva MMCG, et al. Lytic resistance of fibrin containing red blood cells. Arterioscler Thromb Vasc Biol [Internet]. 2011 Oct [cited 2020 Oct 2];31(10):2306–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21737785
21. Cines DB, Lebedeva T, Nagaswami C, Hayes V, Massefski W, Litvinov RI, et al. Clot contraction: compression of erythrocytes into tightly packed polyhedra and redistribution of platelets and fibrin. Blood [Internet]. 2014 Mar 6 [cited 2020 Oct 2];123(10):1596–603. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24335500
22. Martinez MR, Cuker A, Mills AM, Crichlow A, Lightfoot RT, Chernysh IN, et al. Enhanced lysis and accelerated establishment of viscoelastic properties of fibrin clots are associated with pulmonary embolism. Am J Physiol Lung Cell Mol Physiol [Internet]. 2014 Mar 1 [cited 2020 Oct 2];306(5):L397-404. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24414255
23. Xu S, Xu Z, Kim O V, Litvinov RI, Weisel JW, Alber M. Model predictions of deformation, embolization and permeability of partially obstructive blood clots under variable shear flow. J R Soc Interface [Internet]. 2017 [cited 2020 Oct 2];14(136). Available from: http://www.ncbi.nlm.nih.gov/pubmed/29142014

24. Tofler GH, Massaro J, O'Donnell CJ, Wilson PWF, Vasan RS, Sutherland PA, et al. Plasminogen activator inhibitor and the risk of cardiovascular disease: The Framingham Heart Study. Thromb Res [Internet]. 2016 Apr [cited 2020 Oct 6];140:30–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26896607
25. van der Bom JG, de Knijff P, Haverkate F, Bots ML, Meijer P, de Jong PTVM, et al. Tissue Plasminogen Activator and Risk of Myocardial Infarction. Circulation [Internet]. 1997 Jun 17 [cited 2020 Oct 6];95(12):2623–7. Available from: https://www.ahajournals.org/doi/10.1161/01.CIR.95.12.2623
26. Juhan-Vague I, Morange PE, Aubert H, Henry M, Aillaud MF, Alessi MC, et al. Plasma Thrombin-Activatable Fibrinolysis Inhibitor Antigen Concentration and Genotype in Relation to Myocardial Infarction in the North and South of Europe. Arterioscler Thromb Vasc Biol [Internet]. 2002 May 1 [cited 2020 Oct 1];22(5):867–73. Available from: https://www.ahajournals.org/doi/10.1161/01.ATV.0000015445.22243.F4
27. Gorog DA. Prognostic Value of Plasma Fibrinolysis Activation Markers in Cardiovascular Disease. J Am Coll Cardiol [Internet]. 2010 Jun 15 [cited 2020 Sep 13];55(24):2701–9. Available from: https://www.sciencedirect.com/science/article/pii/S0735109710013446?via%3Dihub
28. Sambola A, García Del Blanco B, Ruiz-Meana M, Francisco J, Barrabés JA, Figueras J, et al. Increased von Willebrand factor, P-selectin and fibrin content in occlusive thrombus resistant to lytic therapy. Thromb Haemost [Internet]. 2016 [cited 2020 Sep 15];115(6):1129–37. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26962963
29. Fatah K, Hamsten A, Blomback B, Blomback M. Fibrin gel network characteristics and coronary heart disease: relations to plasma fibrinogen concentration, acute phase protein, serum lipoproteins and coronary atherosclerosis. Thromb Haemost. 1992;68(2):130–5.
30. Fatah K, Silveira A, Tornvall P, Karpe F, Blomback M, Hamsten A. Proneness to formation of tight and rigid fibrin gel structures in men with myocardial infarction at a young age. Thromb Haemost. 1996;76(4):535–40.
31. Bainey KR, Fu Y, Wagner GS, Goodman SG, Ross A, Granger CB, et al. Spontaneous reperfusion in ST-elevation myocardial infarction: Comparison of angiographic and electrocardiographic assessments. Am Heart J [Internet]. 2008 Aug 1 [cited 2020 Sep 15];156(2):248–55. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0002870308002263
32. Fefer P, Hod H, Hammerman H, Boyko V, Behar S, Matetzky S. Relation of Clinically Defined Spontaneous Reperfusion to Outcome in ST-Elevation Myocardial Infarction. Am J Cardiol [Internet]. 2009 Jan 15 [cited 2020 Sep 15];103(2):149–53. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0002914908015099
33. Sumaya W, Wallentin L, James SK, Siegbahn A, Gabrysch K, Bertilsson M, et al. Fibrin clot properties independently predict adverse clinical outcome following acute coronary syndrome: a PLATO substudy. Eur Heart J [Internet]. 2018 [cited 2020 Sep 15];39(13):1078–85. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29390064
34. Sumaya W, Wallentin L, James SK, Siegbahn A, Gabrysch K, Himmelmann A, et al. Impaired Fibrinolysis Predicts Adverse Outcome in Acute Coronary Syndrome Patients with Diabetes: A PLATO Sub-Study. Thromb Haemost [Internet]. 2020 Mar [cited 2020 Sep 15];120(3):412–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/31975352
35. Saraf S, Christopoulos C, Salha I Ben, Stott DJ, Gorog DA. Impaired Endogenous Thrombolysis in Acute Coronary Syndrome Patients Predicts Cardiovascular Death and Nonfatal Myocardial Infarction. J Am Coll Cardiol [Internet]. 2010 May 11 [cited

2020 Sep 17];55(19):2107–15. Available from: https://www.sciencedirect.com/science/article/pii/S0735109710008673
36. Farag M, Spinthakis N, Gue YX, Srinivasan M, Sullivan K, Wellsted D, 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 [Internet]. 2018 Oct 30 [cited 2018 Dec 13];14(40):295–305. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30380032
37. Varin R, Mirshahi S, Mirshahi P, Klein C, Jamshedov J, Chidiac J, et al. Whole blood clots are more resistant to lysis than plasma clots--greater efficacy of rivaroxaban. Thromb Res [Internet]. 2013 Mar 1 [cited 2020 Sep 15];131(3):e100-9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23313382
38. Mega JL, Braunwald E, Wiviott SD, Bassand J-P, Bhatt DL, Bode C, et al. Rivaroxaban in Patients with a Recent Acute Coronary Syndrome. N Engl J Med [Internet]. 2012 Jan 5 [cited 2019 Jan 13];366(1):9–19. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22077192
39. Eikelboom JW, Connolly SJ, Bosch J, Dagenais GR, Hart RG, Shestakovska O, et al. Rivaroxaban with or without Aspirin in Stable Cardiovascular Disease. N Engl J Med [Internet]. 2017 Oct 5 [cited 2019 Jan 14];377(14):1319–30. Available from: http://www.nejm.org/doi/10.1056/NEJMoa1709118
40. Sporns P, Hanning U, Schwindt W, Velasco A, Buerke b, Cnyrim C, et al. Ischemic Stroke: Histological Thrombus Composition and Pre-Interventional CT Attenuation Are Associated with Intervention Time and Rate of Secondary Embolism. Cerebrovasc Dis [Internet]. 2017 [cited 2020 Sep 16];44(5–6). Available from: https://pubmed.ncbi.nlm.nih.gov/29130956/
41. Alessi M-C, Gaudin C, Grosjean P, Martin V, Timsit S, Mahagne M-H, et al. Changes in Activated Thrombin-Activatable Fibrinolysis Inhibitor Levels Following Thrombolytic Therapy in Ischemic Stroke Patients Correlate with Clinical Outcome. Cerebrovasc Dis [Internet]. 2016 [cited 2020 Sep 16];42(5–6):404–14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27387478
42. Kim SH, Han SW, Kim EH, Kim DJ, Lee KY, Kim DI, et al. Plasma fibrinolysis inhibitor levels in acute stroke patients with thrombolysis failure. J Clin Neurol [Internet]. 2005 Oct [cited 2020 Sep 16];1(2):142–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20396461
43. Bembenek JP, Niewada M, Siudut J, Plens K, Członkowska A, Undas A. Fibrin clot characteristics in acute ischaemic stroke patients treated with thrombolysis: the impact on clinical outcome. Thromb Haemost [Internet]. 2017 [cited 2020 Sep 15];117(7):1440–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28382369
44. Guimarães AHC, de Bruijne ELE, Lisman T, Dippel DWJ, Deckers JW, Poldermans D, et al. Hypofibrinolysis is a risk factor for arterial thrombosis at young age. Br J Haematol [Internet]. 2009 Apr [cited 2020 Sep 16];145(1):115–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19183334
45. Drabik L, Konieczyńska M, Undas A. Clot Lysis Time Predicts Stroke During Anticoagulant Therapy in Patients with Atrial Fibrillation. Can J Cardiol [Internet]. 2020 Jan [cited 2020 Sep 16];36(1):119–26. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0828282X19311432
46. Taomoto K, Ohnishi H, Kuga Y, Nakashima K, Ichioka T, Kodama Y, et al. Platelet Function and Spontaneous Thrombolytic Activity of Patients with Cerebral Infarction Assessed by the Global Thrombosis Test. Pathophysiol Haemost Thromb [Internet]. 2009 [cited 2020 Oct 2];37(1):43–8. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/20516672
47. Stanford SN, Sabra A, Lawrence M, Morris RHK, Storton S, Wani M, et al. Prospective evaluation of blood coagulability and effect of treatment in patients with stroke using rotational thromboelastometry. J Stroke Cerebrovasc Dis [Internet]. 2015 Feb [cited 2020 Sep 16];24(2):304–11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25498737
48. McDonald MM, Wetzel J, Fraser S, Elliott A, Bowry R, Kawano-Castillo JF, et al. Thrombelastography does not predict clinical response to rtPA for acute ischemic stroke. J Thromb Thrombolysis [Internet]. 2016 Apr [cited 2020 Sep 16];41(3):505–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26407682
49. Lisman T, de Groot PG, Meijers JCM, Rosendaal FR. Reduced plasma fibrinolytic potential is a risk factor for venous thrombosis. Blood [Internet]. 2005 Feb 1 [cited 2020 Sep 17];105(3):1102–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15466929
50. Karasu A, Baglin TP, Luddington R, Baglin CA, van Hylckama Vlieg A. Prolonged clot lysis time increases the risk of a first but not recurrent venous thrombosis. Br J Haematol [Internet]. 2016 Mar 1 [cited 2020 Sep 17];172(6):947–53. Available from: http://doi.wiley.com/10.1111/bjh.13911
51. Meltzer ME, Lisman T, Doggen CJM, de Groot PG, Rosendaal FR. Synergistic effects of hypofibrinolysis and genetic and acquired risk factors on the risk of a first venous thrombosis. PLoS Med [Internet]. 2008 May 6 [cited 2020 Sep 17];5(5):e97. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18462012
52. Traby L, Kollars M, Eischer L, Eichinger S, Kyrle PA. Prediction of recurrent venous thromboembolism by clot lysis time: a prospective cohort study. PLoS One [Internet]. 2012 [cited 2020 Sep 12];7(12):e51447. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23240024
53. Undas A, Cieśla-Dul M, Drążkiewicz T, Sadowski J. Altered fibrin clot properties are associated with residual vein obstruction: effects of lipoprotein(a) and apolipoprotein(a) isoform. Thromb Res [Internet]. 2012 Sep [cited 2020 Oct 2];130(3):e184-7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22762941
54. Morris TA, Marsh JJ, Chiles PG, Auger WR, Fedullo PF, Woods VL, et al. Fibrin derived from patients with chronic thromboembolic pulmonary hypertension is resistant to lysis. Am J Respir Crit Care Med [Internet]. 2006 Jun 1 [cited 2020 Oct 2];173(11):1270–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16514114
55. Harahsheh Y, Ho KM. Use of viscoelastic tests to predict clinical thromboembolic events: A systematic review and meta-analysis. Eur J Haematol [Internet]. 2018 Feb 1 [cited 2020 Sep 12];100(2):113–23. Available from: http://doi.wiley.com/10.1111/ejh.12992
56. Moore HB, Moore EE, Liras IN, Gonzalez E, Harvin JA, Holcomb JB, et al. Acute Fibrinolysis Shutdown after Injury Occurs Frequently and Increases Mortality: A Multicenter Evaluation of 2,540 Severely Injured Patients. J Am Coll Surg [Internet]. 2016 Apr [cited 2020 Sep 17];222(4):347–55. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26920989
57. Raza I, Davenport R, Rourke C, Platton S, Manson J, Spoors C, et al. The incidence and magnitude of fibrinolytic activation in trauma patients. J Thromb Haemost [Internet]. 2013 Feb [cited 2020 Sep 17];11(2):307–14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23176206
58. Creel-Bulos C, Auld SC, Caridi-Scheible M, Barker N, Friend S, Gaddh M, et al. Fibrinolysis Shutdown and Thrombosis in A COVID-19 ICU. Shock [Internet]. 2020 Aug 4 [cited 2020 Sep 17]; Available from:

https://journals.lww.com/10.1097/SHK.0000000000001635
59. Nougier C, Benoit R, Simon M, Desmurs-Clavel H, Marcotte G, Argaud L, et al. Hypofibrinolytic state and high thrombin generation may play a major role in SARS-COV2 associated thrombosis. J Thromb Haemost [Internet]. 2020 Sep 11 [cited 2020 Sep 17];18(9):2215–9. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/jth.15016
60. Wright FL, Vogler TO, Moore EE, Moore HB, Wohlauer M V, Urban S, et al. Fibrinolysis Shutdown Correlation with Thromboembolic Events in Severe COVID-19 Infection. J Am Coll Surg [Internet]. 2020 [cited 2020 Nov 30];231(2):193-203.e1. Available from: http://www.ncbi.nlm.nih.gov/pubmed/32422349

Table 1. Summary of the advantages and disadvantages of available methods to assess endogenous fibrinolysis.

Method	Advantages	Disadvantages
Factorial Assays	• Convenient for population analyses	• Measurement of one activator/inhibitor does not give an accurate representation of overall fibrinolytic function, which is governed by many such proteins. • Different proteins have varying magnitude of effect • Measuring total protein vs activated protein will have different implications • Total and activated protein levels will differ during normal states and times of thrombus formation. • No conclusive prognostic data • No information on cell-mediated fibrinolytic effects
Euglobulin Clot Lysis (ECL)		• Long preparation time requiring expertise • Blood sample collected into citrated bottles, impacting coagulation • Non-physiological, owing to the removal of cellular components, and fibrinolysis inhibitors • Clot formation stimulated by exogenous activator • Results not obtained in a clinically relevant time frame
Plasma Clot Lysis (CLT)	• Provides prognostic data	• Long preparation time requiring expertise • Employs citrated blood, impacting coagulation • Non-physiological, owing to the removal of cellular components • Clot formation stimulated by exogenous activators, under static conditions • Fibrinolysis initiated by exogenous plasminogen activator • Results not obtained in a clinically relevant time frame
Global Thrombosis Test (GTT)	• Uses whole blood • Platelet activation secondary to high shear, mimicking an endogenous luminal narrowing • Not reliant on exogenous activators • Easy to use, automated, point-of-care test • Results obtained in a clinically relevant time frame: 1-2 hours • Measures platelet reactivity in addition to endogenous fibrinolysis	• Uses non-anticoagulated blood, and therefore sample should be inserted into instrument immediately on blood draw • Not relevant to venous thrombus

	• Provides prognostic data	
Viscoelastic tests (TEG and ROTEM)	• Global assessment of thrombosis and endogenous fibrinolysis • Uses native or citrated blood • Automated • Can be used at the point-of-care (native blood), or laboratory (citrated blood) • Not reliant on exogenous activators (although can be used to facilitate a quick result)	• Only relevant to venous thrombosis, not to arterial thrombosis • In the absence of activators, results can take a few hours

ROTEM: rotational thromboelastometry, TEG: thromboelastometry

Figure legend

Figure 1. Illustration of the pathogenesis of arterial and venous thrombi.

NETS: neutrophil extracellular traps, PAI-1: plasminogen activator inhibitor-1, TAFI: thrombin activatable fibrinolysis inhibitor, t-PA: tissue-plasminogen activator, uPA: urokinase plasminogen activator, vWF: von Willebrand Factor

Figure 2. Relationship of enhanced or impaired endogenous fibrinolysis to clinical events.