Accepted Manuscript.
Article accepted for publication in the Journal of American College of Cardiology, 02/09/2019.

Review Topic of the Week:

Impaired spontaneous (endogenous) fibrinolytic status: Are we ready for a new cardiovascular risk factor?

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Word count 4860 (excl. references)

Funding: There is no funding associated with this manuscript.

Disclosures: DAG is related through family to a company director in Thromboquest Ltd, but neither she, nor her spouse, nor children have financial involvement or equity interest in and have received no financial assistance, support, or grants from the aforementioned. GYHL has nothing to disclose in relation to this manuscript.

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Abstract

Endogenous fibrinolysis is a powerful natural defence mechanism against lasting arterial thrombotic occlusion. Recent prospective studies show that impaired endogenous fibrinolysis (or hypofibrinolysis) can be detected in a significant number of patients with acute coronary syndrome (ACS) using global assays and is a strong marker of future cardiovascular risk. This novel risk biomarker is independent of traditional cardiovascular risk factors and unaffected by antiplatelet therapy. Nevertheless, the optimal test to assess endogenous fibrinolysis is not clear. Most prospective, prognostic data have been obtained using a global assay employing native whole blood at high shear or plasma turbidimetric assays.
For example, tests of endogenous fibrinolysis could be used to identify ACS patients who, despite antiplatelet therapy, remain at high cardiovascular risk. Clinical trials of pharmacotherapy to favourably modulate fibrinolytic status are required as a potential new avenue to improve outcomes in ACS.

Word count 138

Condensed Abstract

Endogenous fibrinolysis is a strong defence mechanism against arterial thrombosis. Impaired endogenous fibrinolysis can be detected in a number of patients with acute coronary syndrome (ACS) and is a marker of future cardiovascular risk. This novel risk factor is independent of traditional cardiovascular risk factors and unaffected by antiplatelet therapy. Tests of endogenous fibrinolysis could be used to identify high-risk ACS patients, who may benefit from additional antithrombotic therapy. The optimal oral pharmacotherapy to improve fibrinolytic profile remains unclear. Clinical trials of pharmacotherapy to favourably modulate fibrinolytic status are required as a potential new avenue to improve outcomes in ACS.

Word count 100

Keywords

fibrinolysis, thrombolysis, acute coronary syndrome, thrombosis, risk factor

Abbreviations

ACS = acute coronary syndrome

AF = atrial fibrillation

A2AP = alpha 2 antiplasmin

CLT = clot lysis time

CVD = cardiovascular disease

DAPT = dual antiplatelet therapy

Hs-CRP = high-sensitivity C-reactive protein

HR = hazard ratio

Lp(a) = lipoprotein (a)

MACE = major adverse cardiovascular events

MI = myocardial infarction

OR = odds ratio

PAI-1= plasminogen activator inhibitor 1

POC = point-of-care

NOAC = non-vitamin K oral anticoagulant

NSTEMI = non-ST elevation myocardial infarction

STEMI = ST-elevation myocardial infarction

TAFI = thrombin activatable fibrinolysis inhibitor

TGA = thrombin generation assay

t-PA = tissue-plasminogen activator

9030 at baseline

Introduction

Occlusive arterial thrombosis is responsible for the majority of cases of myocardial infarction and ischaemic stroke. In acute coronary syndrome (ACS), plaque rupture or erosion results in platelet aggregation and activation of coagulation, leading to thrombosis. At the same time, enzymatic processes that mediate endogenous fibrinolysis serve as a natural defence mechanism to prevent lasting thrombotic occlusion. The balance between pro-aggregatory and pro-fibrinolytic factors determines the outcome of the thrombotic stimulus(1) Myocardial infarction has been termed the failure of timely spontaneous thrombolysis.(2) Some 15% of patients with ST-elevation myocardial infarction (STEMI) exhibit spontaneous reperfusion within an hour after the vascular injury as a consequence of activation of the endogenous fibrinolytic system.(3) Impaired endogenous fibrinolysis is an independent risk factor in ACS,(4) which could be potentially modifiable.

For the assessment of fibrinolysis to be clinically useful, the measurement should be pathophysiologically relevant, easy to perform and interpret, and shown to be predictive of increased risk. The potential for this to provide individual patient risk stratification, over and above current risk factors, and for subsequent pharmacological modulation of fibrinolysis to improve outcomes, would be essential for this to be incorporated into clinical practice. This review provides an overview of currently available tests of fibrinolysis, their relevance to *in vivo* arterial thrombosis (Figure 1), data supporting the clinical usefulness of such tests and potential options for pharmacological modulation of impaired fibrinolysis.

Assessment of endogenous fibrinolysis

i. Factorial (non-global) assays

Assays of individual proteins or factors in the coagulation and fibrinolytic pathways have been studied historically and are summarised below. However, the sheer number of factors involved, coupled with the complexity of interactions, is such that no single marker can fully reflect the overall fibrinolytic profile.

PAI-1 and t-PA

Free tissue plasminogen activator (t-PA) in the circulation immediately forms a complex with circulating plasminogen activator inhibitor 1(PAI-1), such that inactive t-PA/PAI-1 complex level correlates with levels of t-PA antigen and PAI-1 activity. Not only is it difficult to know whether to measure PAI-1 antigen (including free active, inactive and complexed PAI-1) or activity, there is also poor agreement between the results generated by commercial methods for PAI-1 antigen or activity, or t-PA antigen. Although in case-control studies, raised t-PA/PAI-1 complex level,(5) and t-PA antigen have been associated with coronary events,(6,7) in prospective studies, the association between t-PA and development of cardiovascular disease (CVD) was weak.(8) In the Framingham cohort, baseline PAI-1 and t-PA antigen levels correlated with 10-year cardiovascular risk,(9) we also have data in patients with established coronary disease, raised t-PA level(10,11) or PAI-1(12,13) activity was associated with increased cardiovascular risk in some studies, but not in others.(14)

Thrombin activatable fibrinolysis inhibitor (TAFI)

Activated thrombin activatable fibrinolysis inhibitor (TAFI, also known as plasma carboxypeptidase B or carboxypeptidase U) inhibits fibrinolysis by cleaving fibrin, resulting in reduced binding of plasminogen to fibrin, less plasmin formation and reduced

fibrinolysis. Whilst some small studies have shown TAFI activity levels to be linked to cardiovascular risk,(15,16) others have not.(17,18) Yet others suggest an inverse relationship between TAFI activity or antigen level and the occurrence of MI.(18,19) Interpretation is compounded by the fact that TAFI antigen levels do not clearly mirror activity, and by genetic polymorphisms that may account for 25% of TAFI level variability, some of which have been weakly linked to cardiovascular risk.(20)

Complement C3

Raised plasma level of complement C3 is associated with myocardial infarction.(21,22) C3 levels are raised in individuals with type 1 and type 2 diabetes.(23,24) Higher plasma C3 level was associated with more compact fibrin clots, prolonged turbidimetric clot lysis time, and was independent of PAI-1 levels, suggesting that C3 mediates a PAI-1 independent pathway for impaired fibrinolysis in diabetes.(24,25) C3 impaired fibrinolysis in a concentration-dependent manner, both *in vitro* and *ex vivo*.(26) C3 levels fall with optimised glycaemic control(23) and inhibition of C3 with an adhiron homologous to C3 abolished C3-induced prolongation of fibrin clot lysis.(27)

D-Dimer

D-dimer is a measure of fibrin breakdown and ongoing fibrinolysis. Whilst some large prospective studies have shown D-dimer levels to predict future cardiovascular events,(14) the majority of studies found no predictive value for either cardiovascular events(28)or development of CVD.(14,29) In practice, D-dimer testing should perhaps be restricted to the

exclusion of venous thrombosis. D-dimer has also been used as a surrogate measure of thrombogenesis in Phase II trials testing new antithrombotic agents in atrial fibrillation.(30) D-dimer level may not only reflect thrombosis risk, but also reflect a chronic inflammatory state. In the LIPID study, D-dimer level was shown to predict long-term risk of arterial and venous events, CVD mortality, and non-CVD noncancer mortality and correlated with, but was independent of high sensitivity C-reactive protein (hs-CRP) level.(31) D-dimer assays do not offer superior diagnostic insight than currently available tests, and can only serve to risk stratify patients who have higher risk of adverse events. Unfortunately due to lack of specificity, the biomarker is unlikely to accurately indicate what particular event would occur (whether arterial or venous thrombosis, cancer or cardiovascular mortality) and so cannot be a target for therapy.(32) There are many commercially available D-dimer assays, that differ in the epitope targeted by the antibody, method of capture and detection, instrumentation required, and calibration standard, such that the results with one assay cannot be extrapolated to another.(33,34) Furthermore, the D-dimer level cut-off level is determined by the manufacturer, and it is not equivalent to the upper limit of the reference interval for the assay. Traditionally, the threshold used to define a positive test was intentionally set low to maximize the sensitivity, which comes at the cost of lowering specificity(35). Further, D-dimer levels naturally increase with age such that standardisation of “norm” should be age-specific.(36)

Lipoprotein (a)

The pathologic role of lipoprotein (a), Lp(a), was traditionally considered to lie in its similarity to low density lipoprotein (LDL), causing atherosclerosis. Later studies unmasked the potent inhibition of fibrinolysis by Lp(a),(37) an effect attributable to the remarkable homology between apo(a), the distinguishing protein component of Lp(a), and the fibrinolytic

proenzyme, plasminogen. Lp(a) can competitively inhibit t-PA-mediated plasminogen activation, and elevated levels are associated with increased clot density and resistance to endogenous thrombolysis(38)

Indeed, there is a strong association between Lp(a) concentration and the occurrence of MI and stroke. A meta-analysis of 126,634 participants in 36 prospective studies showed that Lp(a) concentration was associated with cardiovascular risk, independent of traditional risk factors,(39) which increased non-linearly with increasing Lp(a) concentrations to a relative risk of 1.9 for individuals with Lp(a) concentration between 77-117mg/dL (90–95th percentile) and 2.60 for those above 117 mg/dL (>95th percentile). Some 25-35% of the population carry small apo(a) isoforms, and such persons who are also exposed to high Lp(a) concentration are at markedly increased cardiovascular risk.(40) A meta-analysis of 40 studies with 58,000 participants showed that persons with smaller apo(a) isoforms have double the risk of CVD compared to those with large isoforms. In secondary prevention, among patients with LDL-C levels <100 mg/dL, elevated Lp(a) is an independent predictor of CVD. Although strong epidemiological evidence suggests that Lp(a) is the single most common genetically-inherited risk factor for premature CVD and an independent risk factor for adverse cardiovascular events, large studies in secondary prevention indicate only a modest association between Lp(a) level and future cardiovascular risk.(41,42)

Thus, although Lp(a) is clearly an independent marker of cardiovascular risk, in secondary prevention and in well-managed statin-treated populations it generally cannot be used for any routine purpose except baseline cardiovascular risk assessment. Furthermore, it is not only LDL and Lp(a), but the extent of oxidation of LDL and Lp(a) that significant impacts on fibrinolysis. The extent of oxidation strongly relates to changes in fibrin clot characteristics *in vitro*, with increased oxidation resulting in more compact networks and increased CLT.(43,44)

ii. Global tests

Static tests

Plasma turbidimetric assays

This laboratory technique can be used to quantify clot formation and structure, and separately, quantify clot lysis time (CLT), as a measure of plasma fibrinolytic potential (Table 1).(45) Assays are performed on citrated plasma in 96-well plates, where changes in optical density that occur with fibrin clot formation and subsequent lysis, are read in a dedicated plate reader. Coagulation is initiated by the addition of a mix that includes tissue factor or thrombin, and t-PA to initiate fibrinolysis. Thus, such tests do not truly represent endogenous fibrinolytic potential, but rather assess the response to extrinsic fibrinolysis with t-PA. The Global Fibrinolytic Capacity is a newer assay, assessing citrated plasma in a micro-cuvette, in a dedicated instrument assessing light transmittance.(46)

The introduction of fluorogenic substrates has developed from thrombin generation assays (TGAs), but fibrinolysis is explored by measuring simultaneous thrombin and plasmin generation through fluorescence to reflect the relationships between clotting and fibrinolysis.(47–49) In particular, like TGA, they have the advantage of permitting calculation of molar concentrations of thrombin and plasmin developed during clotting and lysis phases. TGA are subject to influences of pre-analytical processing and short storage time, double centrifugation and minimising freeze-thaw cycles improves performance.(50) There has been little uptake of such TGA-like methods which remain largely a research tool. However, for all plasma clot lysis assays, the requirement to add excess t-PA makes these methods insensitive to the influence of intrinsic t-PA and subtle variations in the concentration of PAI-1.(48)

Nonetheless, turbidimetric analysis requires trained laboratory personnel, and since results may vary between laboratories, it is not currently suitable as a clinical screening test. There are significant analytical challenges, as well as a need for standardization between laboratories to establish healthy and prothrombotic ranges and facilitate interlaboratory comparisons.(51)

Viscoelastic assays

These techniques, for example, TEG (Haemonetics, Braintree, Massachusetts, USA) or ROTEM (Tem International, GmbH, Munich, Germany) utilise native or citrated whole blood to measure clot formation under very low shear, by transducing changes in the viscoelastic properties of the blood sample, to which a constant rotational force is applied (Table 1). Clotting is initiated with kaolin, with or without tissue factor. A transducer detects changes in clot viscoelastic properties that reflect clot formation and lysis, converting these into electrical signals to create graphical and numerical output. Few studies have found any benefit to using viscoelastic assays for arterial thrombosis risk assessment. Among 270 patients undergoing PCI, patients who experienced adverse cardiovascular events more frequently exhibited higher maximal fibrin clot strength and shorter clot formation time, than patients without events.(52) Such tests are generally not useful for detecting hypofibinolysis associated with arterial thrombosis, and largely used to assess hyperfibrinolysis such as bleeding in trauma and intraoperatively, in particular during cardiac surgery.

Assessment of fibrinolysis under flow

In a plasma clot or even whole blood clot formed under static conditions, the effectiveness of fibrinolysis is determined by the inward rate of diffusion of fibrinolysis activators from

outwith the clot. In the fibrinolysis of an arterial thrombus that is formed under dynamic flow conditions, platelets and flow play critical roles.

Flow chamber devices

Flow chamber devices measure thrombus formation on a pathologically relevant substrate such as collagen or tissue thromboplastin, under adjustable shear rates (Table 1). Thrombus formation and breakdown are quantified by flow pressure changes or fluorescent microscopy of labelled platelets and fibrinogen. Early custom-made flow chambers utilised histological analysis, with the major drawback of requiring large blood volumes, but significantly contributed to the understanding of the significance of flow and shear stress to the dynamics of thrombosis. Subsequently, commercially available microchip-based, microfluidic flow chamber systems have been introduced (Total Thrombus-formation Analysis System T-TAS; Fujimori Kogyo Co. Ltd., Tokyo, Japan; Vena8 Fluoro+BioChips, Cellix Ltd., Ireland).(54,55) Citrate-anticoagulated whole blood, labelled with immunohistochemical stains, is perfused over a microchip coated with collagen and tissue thromboplastin at selected shear rates. Flow-pressure changes signal the onset of thrombus formation and occlusion. Having generated nonocclusive fluorescent thrombi, a second perfusion is performed with blood now supplemented with t-PA, u-PA, or PAI-1, following which fibrinolysis is quantified by confocal microscopic assessment of the reduction in total thrombus and fibrin area. Plasminogen activators and shear-rate prolong occlusion time and reduce total thrombus and fibrin area, which is inhibited by PAI-1. As the developing thrombus is continuously accessible to fibrinolysis activators in the perfusate, these techniques measure external fibrinolysis, mimicking thrombolytic therapy, and not endogenous (spontaneous) fibrinolysis.

Accepted Manuscript.
article accepted for publication in the Journal of Amerrica College of Cardiology, 02/09/2019.

Global assay using native whole blood at high shear

The Global Thrombosis Test (Thromboquest Ltd., London, UK) is an automated, point-of-care technique measuring thrombus formation and lysis from a non-anticoagulated blood sample (Table 1).(56) The thrombogenic stimulus is high shear rate alone, and no chemicals (platelet agonists or pro-coagulants) are added. The blood sample is subjected to high shear, which stimulates occlusive thrombus formation (occlusion time, OT) and this together with spontaneous restart of flow is detected by a photosensor (lysis time, LT). After stabilization of the occlusive thrombi, detection of the first blood drop downstream indicates the onset of thrombolysis, while the number of drops reflects the rate of thrombolysis. The restart of flow after occlusive thrombus formation reflects endogenous thrombolytic activity including fibrinolysis, thrombus stability and contractility. The results are displayed in digital form and simultaneously stored on a memory card, for subsequent review in both numeric and graphic format.

Clinical relevance of measuring endogenous fibrinolysis

Identifying patients at high cardiovascular risk

The importance of assessing endogenous fibrinolysis relates to its ability to identify subjects at increased cardiovascular risk, and the potential to pharmacologically modulate impaired fibrinolysis, to improve outcomes.

Individual factorial markers of fibrinolysis, such as t-PA, PAI-1, TAFI have limited predictive value, and will not be discussed further. Early evidence for impaired endogenous fibrinolysis as a marker of cardiovascular risk came from case control studies, which assessed plasma fibrin clot characteristics and turbidimetric assessment of clot lysis.

Retrospective case control studies have shown that hypofibrinolysis is a risk factor for arterial thrombosis including premature MI.(57,58) Patients at high cardiovascular risk, such as those with diabetes mellitus, chronic kidney disease, stroke, and peripheral arterial disease, tend to form dense *in vitro* fibrin clots with impaired fibrinolysis.(59) A large prospective study in ACS showed that impairment of fibrin clot lysis is associated with adverse clinical outcomes, independent of clinical characteristics and inflammatory status.(60)

Among 421 men with a first MI and 642 controls, risk of MI was 1.4-fold higher for subjects with plasma clot lysis time (CLT) assessed using turbidimetry, in the highest quartile compared with those in the lowest, and risk was particularly pronounced for men <50 years old (odds ratio [OR] 3.2, 95% confidence interval [CI] 1.5-6.7), even after adjustment for cardiovascular risk factors.(57) Compared to healthy controls, young survivors of a first arterial thrombotic event had significantly longer CLT, with a CLT above the 60th, 70th, 80th, 90th and 95th percentiles showing a progressive increase in risk of arterial thrombosis (OR 1.7, 2.0, 2.3, 2.3 and 2.9, respectively).(61) Retrospective studies revealed that patients at increased cardiovascular risk form abnormally dense fibrin thrombi *in vitro*, that are more resistant to fibrinolysis, than patients at low risk. This is well documented, *inter alia*, in patients with diabetes,(62) renal failure,(59) ischaemic stroke(63) and stent thrombosis(64). In the largest case control study of 800 patients with prior MI and 1,123 controls, an abnormally low fibrinolysis activation profile or an abnormally long CLT measured by a global assay, was associated with significantly increased risk of first MI, even after adjustment for traditional cardiovascular risk factors.(65) However, conclusions drawn from case-control studies are limited because of selection bias, a possibility of reverse causality and the assumption that levels of the measured biomarker would correspond to the levels as they were before the ischaemic event occurred.

Prolonged lysis times have been shown to predict adverse events in patients with non-ST elevation myocardial infarction (NSTEMI)(66) and end-stage renal disease.(56) More recently, prolonged lysis time was shown to be highly predictive of recurrent adverse events in patients with STEMI, independent of conventional cardiovascular risk factors.(67)
A *prospective* study in 300 patients with ACS, predominantly NSTEMI, endogenous fibrinolysis was measured using the point-of-care Global Thrombosis Test, on average 5 days post-admission. Impaired endogenous fibrinolysis was detected in 23% of patients despite dual antiplatelet medication (DAPT), and was a significant and independent predictor of major adverse cardiovascular events (MACE, composite of heart attack, stroke and cardiovascular death) at 12 months (hazard ratio 2.52, 95%CI 1.34-4.71, p=0.004).(66) In a prospective study of patients with end-stage renal failure on haemodialysis, impaired endogenous fibrinolysis using the Global Thrombosis Test was detected in 42% of patients and was strongly associated with development of MACE (HR 4.25, 95%CI 1.58-11.46, p=0.004), driven by non-fatal MI and stroke (HR 14.28, 95%CI 1.86-109.90, p=0.01).(56)

The largest prospective study to assess the utility of fibrinolysis assessment on cardiovascular risk assessment, using a global assay, was a substudy of the PLATelet inhibition and patient Outcomes (PLATO) trial.(60) In this multicentre study of 4,354 patients with ACS, endogenous fibrinolysis was assessed on average 6 days after admission, using a turbidimetric assay to determine plasma CLT and maximum turbidity (a measure of clot density). After adjusting for traditional cardiovascular risk factors and other predictive markers, each 50% increase in lysis time was associated with a 20% relative increase in cardiovascular death (HR 1.2; 95%CI 1.01-1.42) and a 21% relative increase in all-cause death (HR 1.2; 95%CI 1.03-1.42) at 1 year. After adjustment for other prognostic biomarkers,

the association with cardiovascular death remained significant for lysis time (HR 1.2, 95%CI 1.01-1.42; p=0.042) but not for maximum turbidity.

The recently published RISK-PPCI study assessed 496 patients with STEMI upon arrival, immediately before PPCI, using the point-of-care Global Thrombosis Test.(67) Impaired endogenous fibrinolysis, detected in 14% of patients, was highly predictive of recurrent MACE (HR 9.1, 95%CI 5.29-15.75; p<0.001), driven by cardiovascular death (HR 18.5, 95%CI 7.69-44.31; p<0.001) and MI (HR 6.2, 95% CI 2.64-14.73; p<0.001), particularly within the first 30 days. Cardiovascular risk increased inversely with effectiveness of fibrinolysis. Fibrinolysis remained strongly predictive of MACE after adjustment for conventional risk factors (HR 8.03, 95%CI 4.28-15.03; p<0.001).

However, global tests, particularly those that employ flow, do not reflect changes that occur in the microenvironment downstream, that includes the formation of downstream microvascular thrombi which contribute to ischaemic injury even after apparent restoration of arterial flow. Cell surface fibrinolysis, namely fibrinolysis on the surfaces of endothelial cells or monocytes offers a second line of fibrinolytic defence, because unlike circulating plasmin, t-PA, and u-PA that are neutralized by circulating inhibitors, several cell surface molecules, including plasminogen receptors and the annexin A2 complex bind plasminogen and t-PA on endothelial cells to promote fibrinolysis.(68,69) Recent animal studies indicate that α2-antiplasmin (a2AP) impairs dissolution of thrombus, and mediates the development of microvascular thrombosis, whereas use of a2AP–inactivating monoclonal antibody diminished microvascular thrombosis.(70)

Detection of hyperfibrinolysis to asses bleeding risk

Massively enhanced fibrinolysis can result in bleeding diatheses. Causes include rare primary congenital hyperfibrinolytic states due to deficiency or overexpression of the components of the fibrinolytic system or secondary hyperfibrinolysis associated with bleeding in clinical settings that include trauma, malignancy, liver failure or surgery-related bleeding associated with liver transplantation or cardiopulmonary bypass. Hyperfibrinolysis is best detected with the euglobulin lysis time or viscoelastic assays (TEG or ROTEM) (48). However, a Cochrane review raised concerns about the accuracy of these assays for bleeding prediction, recommending their use be confined to research.(53)

Plasma turbidity assays, which employ the addition of t-PA are clearly insensitive to the effects of t-PA level. The global fibrinolytic capacity has the advantage that, since no fibrinolysis activators are added, it is sensitive for abnormalities in t-PA, PAI-1, fibrinogen and TAFI activity. The treatment of hyperfibrinolysis involves correcting underlying causes and use of fibrinolysis inhibitors such as epsilon-aminocaproic acid or tranexamic acid.(71)

The optimal fibrinolysis level using global tests has not been defined, but hyperfibrinolysis is relatively rare and hypofibrinolysis relatively common. Outside of massive bleeding, a shorter fibrinolysis time appears beneficial and associated with reduced cardiovascular risk.

Potential pharmacologic modulation of fibrinolysis

Fibrinolysis is clearly modulated by intravenous fibrinolytic/thrombolytic therapy, which remains a therapeutic treatment for STEMI and ischaemic stroke and will not be discussed.

Drugs that directly target platelet activation or coagulation

Aspirin

Studies *in vitro* have shown that aspirin renders the fibrin networks looser and prolongs CLT.(72) Perfusion of aspirin-treated blood over endothelium-denuded rabbit aorta in a perfusion chamber had no effect on thrombus volume but enhanced thrombus fragility and fragmentation.(73,74)

$P2Y_{12}$ receptor antagonists

In addition to inhibiting platelet aggregation and thrombus growth, $P2Y_{12}$ inhibitors reduce thrombus stability and induce thrombus fragmentation *in vitro* and in animal models.(75,76) In clinical studies, both the PLATO substudy using plasma clot lysis(60) and the RISK-PPCI study using the Global Thrombosis Test(67) showed that endogenous fibrinolytic status was unaffected by DAPT, regardless of whether clopidogrel or ticagrelor was used.(60)
In patients with CVD, measurement of endogenous fibrinolysis in whole blood using the Global Thrombosis Test before and during clopidogrel, ticagrelor or cangrelor treatment showed that all $P2Y_{12}$ receptor antagonists resulted in thrombus instability *in vitro* but only cangrelor significantly enhanced endogenous fibrinolysis, although a trend was seen for ticagrelor.(77) *In vitro*, cangrelor had no effect on PAI-1 release from platelet alpha granules, but in combination with aspirin, it very significantly reduced the PAI-1 release induced by collagen(78)

Glycoprotein IIb/IIIa inhibitors

In addition to the inhibition of platelet aggregation, glycoprotein IIb/IIIa inhibitors promote instability of pre-formed thrombi by enhancing platelet disaggregation. Abciximab added to human blood dose-dependently enhanced the disaggregation of platelet thrombus formed on collagen under flow conditions *in vitro*(79) increased clot permeability and susceptibility to

fibrinolysis.(80) Perfusion of human blood containing abciximab, eptifibatide, or tirofiban over freshly formed thrombus *in vitro* caused thrombus dissolution, through fragmentation,(80) an effect that may be mediated through dissociation of fibrinogen from the platelet surface.

Non-vitamin K antagonist oral anticoagulants (NOACs)

Spiking human plasma with NOACs resulted in accelerated turbidimetric plasma clot lysis in response to t-PA(81,82) and lysis time was inversely correlated with NOAC concentration.(83) Plasma clot lysis *in vitro* is enhanced by all NOACs; dabigatran, apixaban, rivaroxaban and edoxaban, through what is likely to be a combination of TAFI-mediated and TAFI-independent mechanisms.(81,84)(82) The rate of clot lysis measured by a micro-plate assay was most rapid in apixaban, then dabigatran, and slowest in rivaroxaban-treated patients with atrial fibrillation (AF).(85) Dabigatran and rivaroxaban both enhanced the susceptibility of human *in vitro* plasma clots to thrombolytic therapy.(86,87) Using the Global Thrombosis Test to assess the effect of NOACs on endogenous fibrinolysis of whole blood in patients with AF, apixaban, rivaroxaban and dabigatran all exhibited a trend toward enhancing endogenous fibrinolysis under high shear conditions *in vitro*.(88) In a prospective cross-sectional analysis of 180 subjects with non-valvular AF, endogenous fibrinolysis time assessed using the Global Thrombosis Test was shorter in patients taking apixaban compared to warfarin or aspirin.(89) A prospective longitudinal study of 80 patients with non-valvular AF assessed before and during apixaban treatment using the Global Thrombosis Test showed that apixaban significantly improved endogenous fibrinolysis with maximal effect in those with impaired fibrinolysis pre-treatment.(89) A pro-fibrinolytic effect of NOACs could underlie the results of the ATLAS ACS 2-TIMI-51(90) and the COMPASS studies,(91) in

which addition of low-dose rivaroxaban to DAPT significantly reduced the risk of adverse cardiovascular events, albeit at a cost of increased bleeding.

Drugs with indirect pleiotropic effects

Statins

Statins exert numerous pleiotropic effects that include enhancement of fibrinolysis and inhibition of platelet activation and coagulation.(92) These effects may in part be indirectly mediated through raising HDL and reducing Lp(a).(92) Statins also stimulate fibrinolysis through reduction in tissue factor (TF) activity, conversion of prothrombin to thrombin and thrombin activity(92–94) and through reduction in PAI-1 and increase in t-PA production.(92,95) In the Multi-Ethnic Study of Atherosclerosis, a healthy subjects who were statin users had significantly lower FVIII levels than non-statin users.(96)

PCSK 9 inhibitors

There is abundant data that PCSK 9 plasma levels relate to enhanced platelet reactivity,(97) and also regulate coagulation as shown by a correlation with plasma TF levels,((98) enhanced thrombin–antithrombin complexes and reduced protein C level.(99) PCSK 9 inhibitors reduce Lp(a) and may contribute to enhanced fibrinolysis.(100,101) The effects of statins and PCSK9 inhibitors may also be modulated through raising HDL levels. HDL inhibits platelet fibrinogen binding and aggregation in response to thrombin, downregulates the coagulation and stimulates fibrinolysis.(102)

Potential future antithrombotic drugs

A number of novel antithrombotic drugs are in development,(103) some with effects on the fibrinolytic system.

Factor XI inhibition

In animal models, FXI inhibition enhanced thrombolysis and treatment with FXI antisense oligonucleotides significantly attenuated thrombus formation and fibrin deposition, with formation of unstable thrombi.(104) The subcutaneous FXI-directed antisense oligonucleotide IONIS-416858 has shown safety in a phase 2 study in humans.(104)

Factor XII inhibition

Factor XII inhibition reduces clot firmness, and thrombi created in FXII-deficient mice are unstable and prone to embolization.(105) In a carotid injury model, recombinant fully human FXIIa activity neutralizing antibody (3F7) dose-dependently reduced thrombus formation at arterial shear rates without an increase bleeding.(106)

TAFI inhibition

DS-1040 is a novel compound that inhibits the activated TAFI. In animal studies DS-1040 enhanced endogenous fibrinolysis and in a first-in-human phase 1 study, intravenous infusion of DS-1040 caused dose-dependent decrease in both TAFIa activity and in CLT.(107)

Endogenous fibrinolysis as a modifiable cardiovascular risk factor

Endogenous fibrinolysis lends itself to cardiovascular risk assessment and screening, and generally conforms to the Wilson–Jungner requirements(108) for a meaningful risk marker since (1) it has biological plausibility, (2) impaired fibrinolysis is associated with increased

cardiovascular risk, (3) impaired fibrinolysis precedes the event, (4) there is evidence of a dose–response relationship between the degree of impairment in fibrinolysis and outcome, (5) there are suitable screening tests and (6) the test would be acceptable to patients.

Finally, (7) there should be an accepted treatment for patients with recognized disease. The last criterion has yet to be defined. *In vitro* studies show that a number of medications can modulate fibrinolysis but of the currently available agents, NOACs show most promise. This is indirectly supported by clinical studies showing that anticoagulation, in addition to antiplatelet therapy, can reduce cardiovascular risk in patients with stable disease or ACS, albeit at the cost of increased bleeding.(90,91) Whilst such a strategy clearly cannot be recommended for all ACS patients, assessing endogenous fibrinolysis could identify those at highest risk, who may benefit most from anticoagulation. In order for endogenous fibrinolysis assessment to translate into an improvement in clinical outcome, large prospective trials are now needed to assess whether “personalised” antithrombotic therapy to enhance fibrinolysis in ACS patients can reduce future cardiovascular events.

Conclusions

Impaired endogenous fibrinolysis can be detected in a significant number of patients with ACS and is a marker of future cardiovascular events. This may be a novel risk factor, largely independent of traditional cardiovascular risk factors and endogenous fibrinolytic status appears largely unaffected by DAPT.

Nevertheless, the best clinical test to assess endogenous fibrinolysis is not clear. Most prospective, prognostic data showing the importance of endogenous fibrinolysis as a marker of cardiovascular risk have been obtained using a global assay employing native whole blood at high shear or plasma turbidimetric assays (Table 1). Optimal physiological assessment of

endogenous fibrinolysis would necessitate the measurement of spontaneous lysis of an autologous thrombus formed from whole blood under flow conditions, without the addition of external fibrinolytics. This would take account of the contribution of flow/shear, of adherent neutrophils to fibrinolysis, and the contribution of platelets, including through generation of thrombin and release of thrombin, PAI-1, TAFI and alpha 2 antiplasmin to both thrombus formation and lysis. Although some current techniques use whole blood, not all assess fibrinolysis under flow conditions, and many assess the response to potentiation of thrombolysis (t-PA) rather than spontaneous fibrinolysis.

Whilst global whole blood tests under high shear are more physiological, they are limited by the inconvenience of short time window of testing due to the use of non-anticoagulated blood. Turbidimetric tests on the other hand employ only plasma, do not take account of other blood constituents or the effects of flow, and employ external agonists.

There is potential to roll these tests out to identify ACS patients who, despite DAPT, remain at high cardiovascular risk. The optimal oral therapy to improve fibrinolytic profile remains to be determined, but NOACs show early promise. Future clinical trials of pharmacotherapy to favourably modulate impaired fibrinolytic status are required as a potential new avenue to improve outcomes for ACS patients.

Main messages of the review

- Endogenous fibrinolysis is a powerful natural defence mechanism against thrombosis.
- Impaired endogenous fibrinolysis in ACS patients is a strong independent marker of increased cardiovascular risk.
- Patients with impaired fibrinolysis may benefit from additional antithrombotic treatment.
- Pharmacological enhancement of fibrinolysis may be a new avenue to improve outcomes in ACS.

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Figure 1 (Central Illustration).

Determinants of endogenous thrombolytic activity and how these are assessed by test of fibrinolysis/thrombolysis.

Arterial thrombus formation involves platelet aggregation and activation of coagulation and fibrinolysis pathways. Lysis of the thrombus is determined by plasma fibrinolysis activators including t-PA and u-PA, and fibrinolysis inhibitors such as PAI-1, alpha 2 antiplasmin and TAFI. Cellular components can affect lysis (such as thrombin release from activated platelets and elastase from PMNL), while blood flow can both potentiate thrombosis through high-shear and cause thrombus dislodgement and dispersion.

Tests of clot lysis in plasma or whole blood under static conditions do not assess important determinants of endogenous thrombolysis such as flow, cellular contribution such as thrombin-release from platelets, clot retraction or stability. Thrombolysis tests provide more global assessment of thrombolysis including the contribution of flow and cellular components but less information on plasma fibrin density.

PAI-1= plasminogen activator inhibitor 1, PMNL= polymorphonuclear leucocytes, TAFI=thrombin-activatable fibrinolysis inhibitor, t-PA=tissue plasminogen activator, uPA=urokinase

Table 1. Comparison of global tests of thrombolysis/fibrinolysis.

	Plasma or whole blood	Anti-coagulant	Stimulus to clot/thrombus formation	Flow, Shear rate	Endogenous or Exogenous lysis	External stimulus to initiate fibrinolysis	Fibrinolysis or thrombolysis	Main disadvantage	Useful to assess risk in ACS	Point of Care	Easy to perform by clinician
Turbidity	Plasma	Yes	Tissue factor/ thrombin	None	Exogenous	t-PA	Plasma clot lysis	Complex laboratory test needing specialist expertise; since t-PA is added it is less sensitive to assess endogenous fibrinolytic potential	Yes	No	No
TEG/ROTEM	Plasma or whole blood	Yes and No	Kaolin/tissue factor	Very low, shear rate $0.1s^{-1}$	Endogenous	None	Whole blood clot lysis	Static assay, platelets have limited role, no real data to support use in ACS	No	Yes	Yes
Flow chamber devices	Whole blood	Yes	Collagen/ tissue thrombo-plastin + High shear	High shear $>10,000s^{-1}$	Both	t-PA, u-PA, PA, or PAI-1	Thrombo-lysis	Complex laboratory test needing specialist expertise	No	No	No
Global Thrombosis Test	Whole blood	No	High shear	High shear $>10,000s^{-1}$	Endogenous	None	Thrombo-lysis	Native blood; test should be started <15 s of blood draw	Yes	Yes	Yes