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JACC REVIEW TOPIC OF THE WEEK

Impaired Spontaneous/Endogenous Fibrinolytic Status as New Cardiovascular Risk Factor?

JACC Review Topic of the Week

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ABSTRACT

Endogenous fibrinolysis is a powerful natural defense mechanism against lasting arterial thrombotic occlusion. Recent prospective studies have shown 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. Most prospective prognostic data have been obtained using a global assay using native whole blood at high shear or plasma turbidimetric assays, which are described herein. Tests of endogenous fibrinolysis could be used to identify patients with ACS who, despite antiplatelet therapy, remain at high cardiovascular risk. This review discusses the impact of currently available medications and those in development that favorably modulate fibrinolytic status and may offer a potential new avenue to improve outcomes in ACS. (J Am Coll Cardiol 2019;74:1366–75) Crown Copyright © 2019 Published by Elsevier on behalf of the American College of Cardiology Foundation. All rights reserved.

Arterial thrombosis is responsible for most cases of myocardial infarction (MI) and ischemic 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 defense mechanism to prevent lasting thrombotic occlusion. The balance between pro-aggregatory and pro-fibrinolytic factors determines outcome (1). Approximately 15% of patients with ST-segment elevation myocardial infarction (STEMI) exhibit spontaneous reperfusion as a consequence of activation of the endogenous fibrinolytic system (2). MI has been termed the failure of timely spontaneous thrombolysis (3). Impaired endogenous fibrinolysis is an independent risk factor in ACS (4), which could be potentially modifiable (2,4). For fibrinolysis assessment to be clinically useful, the measurement should be pathophysiologically relevant, easy to perform and interpret, and predictive of risk. The potential for this to provide individual 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. We provide an overview

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HIGHLIGHTS

- Endogenous fibrinolysis is a natural defense against lasting arterial thrombotic occlusion.
- Impaired fibrinolysis is detectable with global assays in a number of patients with ACS and CVD.
- Impaired fibrinolysis is a strong, independent and novel marker of cardiovascular risk.
- Future trials of pharmacotherapy to enhance chronic fibrinolytic status are required to reduce cardiovascular risk.

of currently available tests of fibrinolysis, their relevance to arterial thrombosis (**Central Illustration**), data that support their clinical usefulness, and potential options for pharmacological modulation.

ASSESSMENT OF ENDOGENOUS FIBRINOLYSIS

FACTORIAL (NONGLOBAL) ASSAYS. Assays of individual proteins or factors in the coagulation and fibrinolytic pathways are summarized in the following, but due to the large number of factors involved, coupled with the complexity of interactions, global tests are preferable.

Plasminogen activator inhibitor-1 and tissue plasminogen activator. Free tissue plasminogen activator (t-PA) in the circulation immediately forms a complex with circulating plasminogen activator inhibitor (PAI)-1, such that the inactive t-PA/PAI-1 complex level correlates with t-PA antigen and PAI-1 activity. It is difficult to know whether to measure PAI-1 antigen (including free active, inactive, and complexed PAI-1) or activity, and there is poor agreement between commercial tests for PAI-1 antigen or activity and t-PA antigen. Although in case–control studies, raised t-PA/PAI-1 complex (5) and t-PA antigen were associated with coronary events (6,7) in prospective studies, the association between t-PA and PAI antigen and the development of cardiovascular disease (CVD) was weak (8,9). In patients with coronary disease, raised t-PA level (10,11) or PAI-1 activity (12) was associated with increased cardiovascular risk in some studies, but not in others (13).

Thrombin activatable fibrinolysis inhibitor. Activated thrombin activatable fibrinolysis inhibitor (TAFI) cleaves fibrin, resulting in reduced binding of plasminogen to fibrin, less plasmin formation, and reduced fibrinolysis. Although some small studies have linked TAFI activity to cardiovascular risk (14,15), others have not (16,17). However, others suggest an inverse relationship between TAFI activity or antigen level and the occurrence of MI (17,18). Interpretation is compounded by the fact that TAFI antigen levels do not clearly mirror activity and by genetic polymorphisms that can influence TAFI levels (19).

Complement C3. Raised plasma level of complement C3 is associated with MI (20) and has been documented in individuals with diabetes (21). Higher C3 levels in individuals with diabetes have been associated with more compact fibrin clots, with concentration-dependent prolongation of turbidimetric clot lysis time (CLT), independent of PAI-1 (21,22). C3 levels fall with optimized glycemic control (21), and inhibition with an adhiron homologue abolishes C3-induced prolongation of CLT (23).

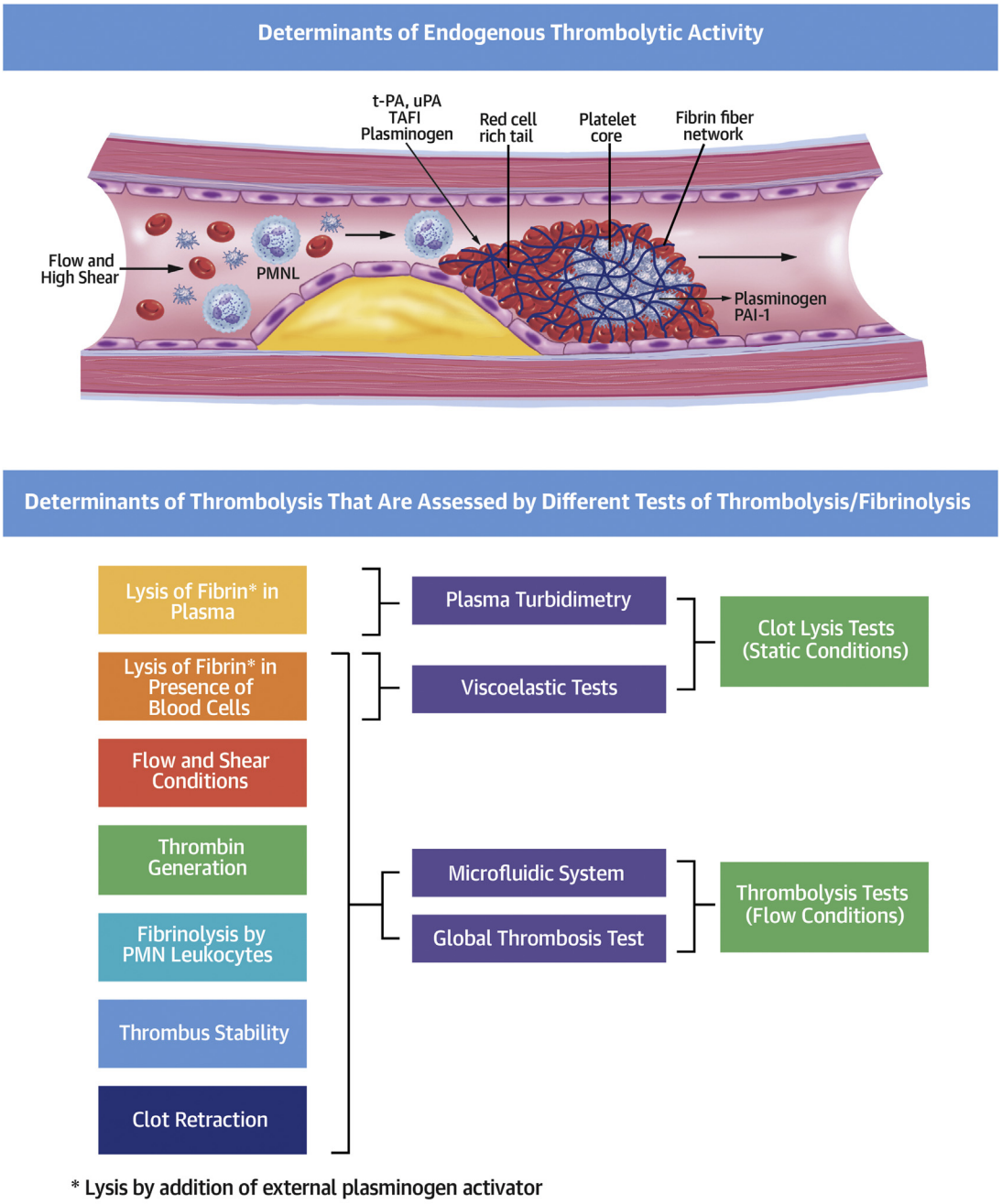
D-dimer. D-dimer is a measure of fibrin breakdown and fibrinolysis, as well as inflammation. Although some large prospective studies have shown D-dimer levels predict future cardiovascular events (13), others have not (13,24). D-dimer levels in 1 study predicted long-term risk of arterial and venous events, cardiovascular and noncancer mortality, and was correlated with, but independent of, high-sensitivity C-reactive protein (25). Although D-dimer level may aid risk stratification, levels increase with age (26) and cannot indicate the type of risk (arterial or venous, cancer or cardiovascular) (27), such that use should be restricted to the exclusion of venous thrombosis. The threshold used to define a positive test is set low to maximize sensitivity, with resultant reduced specificity (28). There are many commercially available assays, differing in target epitope and method of capture, as well as detection, instrumentation, and calibration standard, such that results with 1 assay cannot be extrapolated to another (29).

Lipoprotein(a). The pathological role of lipoprotein (a) [Lp(a)] lies in its similarity to low-density lipoprotein and the potent inhibition of fibrinolysis by Lp(a) (30), an effect attributable to the homology between the apolipoprotein(a) component of Lp(a) and plasminogen. Lp(a) can competitively inhibit t-PA–mediated plasminogen activation, and elevated levels are associated with increased clot density and resistance to thrombolysis (31). Oxidation of

ABBREVIATIONS AND ACRONYMS

ACS = acute coronary syndrome
AF = atrial fibrillation
A2AP = alpha 2 antiplasmin
CI = confidence interval
CLT = clot lysis time
CVD = cardiovascular disease
DAPT = dual antiplatelet therapy
DOAC = direct oral anticoagulant
HR = hazard ratio
Lp(a) = lipoprotein (a)
MACE = major adverse cardiovascular event
MI = myocardial infarction
PAI = plasminogen activator inhibitor
STEMI = ST-segment elevation myocardial infarction
TAFI = thrombin activatable fibrinolysis inhibitor
t-PA = tissue-plasminogen activator
u-PA = urokinase plasminogen activator

CENTRAL ILLUSTRATION **Determinants of Endogenous Thrombolytic Activity and How These Are Assessed by Testing Fibrinolysis and Thrombolysis**



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Arterial thrombus formation involves activation of platelets, coagulation, and fibrinolysis. Lysis of the thrombus is determined by fibrinolysis activators, including tissue-plasminogen activator (t-PA) and urokinase plasminogen activator (u-PA), and fibrinolysis inhibitors such as plasminogen activator inhibitor 1 (PAI-1), alpha 2 antiplasmin (A2AP), and thrombin activatable fibrinolysis inhibitor (TAFI). Cellular components affect lysis (e.g., thrombin release from activated platelets and elastase from polymorphonuclear leucocytes [PMNLs]), whereas blood flow can both potentiate thrombosis through high shear and cause dislodgement and dispersion. Clot lysis tests under static conditions do not assess important determinants of endogenous thrombolysis such as flow, cellular contributions, clot retraction, or stability. Thrombolysis tests provide more global assessment, including the contribution of flow and cellular components but less information on plasma fibrin density.

TABLE 1 Comparison of Global Tests of Thrombolysis and/or 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; because 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 0.1 s^{-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 >10,000 s^{-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 >10,000 s^{-1}	Endogenous	None	Thrombo-lysis	Native blood; test should be started <15 s of blood draw	Yes	Yes	Yes

ACS = acute coronary syndrome; PA = plasminogen activator; PAI-1= plasminogen activator inhibitor 1; t-PA = tissue-plasminogen activator; u-PA = plasminogen activator.

low-density lipoprotein and/or Lp(a) further increases clot density and CLT (32). Lp(a) is an independent cardiovascular risk factor (33), and risk is nonlinearly related to Lp(a) levels. Individuals with high Lp(a) who also carry smaller apolipoprotein(a) isoforms have a 2-fold cardiovascular risk compared with those with large isoforms (34). In individuals with low-density lipoprotein cholesterol <100 mg/dl, elevated Lp(a) is an independent predictor of CVD. Although epidemiological evidence suggests that Lp(a) is the most common genetically inherited risk factor for premature CVD, large studies in secondary prevention have indicated only a modest association with cardiovascular risk (35,36). Furthermore, Lp(a) level remains largely unchanged through life, so it is only useful for baseline risk assessment.

GLOBAL TESTS

STATIC TESTS. Plasma turbidimetric assays. This laboratory technique can be used to quantify clot formation and structure, and separately, quantify CLT, as a measure of plasma fibrinolytic potential (**Table 1**) (37). 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 they assess the response to extrinsic fibrinolysis with t-PA. Because citrated plasma rather than whole blood is used, it does not assess the contribution of platelets or neutrophils to thrombosis or fibrinolysis. The Global Fibrinolytic Capacity, a newer assay, assesses citrated plasma in a micro-cuvette, in a dedicated instrument that assesses light transmittance (38). The option of using fluorogenic substrates stems from thrombin generation assays and allows simultaneous measure of molar concentrations of thrombin and plasmin generation through fluorescence, reflecting relationships between clotting and fibrinolysis (39,40). However, it is subject to pre-analytical influences such as storage, centrifugation, and freeze–thaw cycles (41), and remains largely a research tool. Furthermore, the requirement to add external t-PA in plasma clot lysis assays makes these methods insensitive to variations in intrinsic t-PA and PAI-1 concentrations (40).

Turbidimetric analysis requires trained laboratory personnel, and because results may vary between laboratories, it is not currently suitable as a clinical screening test. There are also significant analytical challenges and need for standardization and establishment of normal ranges to facilitate interlaboratory comparisons (42).

Viscoelastic assays. Techniques such as TEG (Haemonetics, Braintree, Massachusetts) or ROTEM

(Tem International, GmbH, Munich, Germany) use native or citrated whole blood to measure clot formation under low shear, by transducing changes in the viscoelastic properties of blood, for 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. These techniques are not useful for assessing hypofibrinolysis and thrombosis risk, but are used to assess hyperfibrinolysis in bleeding conditions (e.g., trauma and during cardiac surgery) (1,43).

ASSESSMENT OF FIBRINOLYSIS UNDER FLOW. In a plasma or whole blood clot formed under static conditions, the effectiveness of fibrinolysis is determined by the inward rate of diffusion of fibrinolysis activators from outside the clot, whereas in the fibrinolysis of an arterial thrombus formed under dynamic flow conditions, platelets and flow play the 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 labeled platelets and fibrinogen. Early custom-made flow chambers required large blood volumes and used histological analysis, but contributed significantly to the understanding of the importance of flow and shear stress in arterial thrombosis. Subsequently, commercially available microchip-based microfluidic flow chamber systems were introduced (Total Thrombus-formation Analysis System T-TAS, Fujimori Kogyo Co. Ltd., Tokyo, Japan; Vena8 Fluoro+BioChips, Cellix, Ireland) (44,45). Citrate-anticoagulated whole blood, labeled 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, urokinase-plasminogen activator (u-PA), or PAI-1, after which fibrinolysis is quantified by confocal microscopic assessment of total thrombus and the fibrin area. Plasminogen activators and shear rate prolong occlusion time and reduce total thrombus and fibrin area, which is inhibited by PAI-1. Because 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.

Global assay using native whole blood at high shear. The Global Thrombosis Test (Thromboquest Ltd., London, United Kingdom) is an automated, point-of-care technique that measures thrombus formation and lysis from a non-anticoagulated blood sample (Table 1) (46). 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), and this, together with spontaneous restart of flow, is detected by a photosensor (lysis time). After stabilization of the occlusive thrombi, detection of the first blood drop downstream indicates the onset of thrombolysis, whereas 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, in numerical and graphic formats.

CLINICAL RELEVANCE OF MEASURING ENDOGENOUS FIBRINOLYSIS

IDENTIFYING SUBJECTS 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, and 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 that assessed plasma fibrin clot characteristics and clot lysis with turbidimetry. Retrospective case–control studies showed that hypofibrinolysis was a risk factor for arterial thrombosis, including premature MI (47,48). In 1 study, risk of MI was 1.4-fold higher for subjects with CLT in the highest quartile compared with those in the lowest, especially for men older than 50 years old (odds ratio: 3.2; 95% confidence interval [CI]: 1.5 to 6.7), even after adjustment for cardiovascular risk factors (47). Similarly, young survivors of a first arterial thrombotic event had significantly longer CLT than healthy control subjects, with longer CLT related to a progressive increase in arterial thrombosis risk (49). In the largest case–control study of 800 patients with previous MI and 1,123 control subjects, an abnormally low fibrinolysis activation profile or an abnormally

long CLT, measured by a global assay, was associated with a significantly increased risk of MI, even after adjustment for traditional cardiovascular risk factors (50). Retrospective studies revealed that individuals at high cardiovascular risk tended to form abnormally dense in vitro fibrin clots with impaired fibrinolysis (51). This was well documented, inter alia, in patients with diabetes (21,51), ischemic stroke (51,52), and stent thrombosis (51). However, conclusions drawn from case–control studies were limited because of selection bias, a possibility of reverse causality, and the assumption that levels of the measured biomarker would correspond to the levels they were before the ischemic event occurred.

Prolonged lysis times were also shown to prospectively predict adverse cardiovascular events. (46,53-55) In a prospective study of 300 patients with ACS, predominantly non-STEMI, endogenous fibrinolysis was measured using the point-of-care Global Thrombosis Test an average 5 days post-admission. Impaired endogenous fibrinolysis, detected in 23% of patients, was a significant and independent predictor of major adverse cardiovascular events (MACEs; a composite of heart attack, stroke, and cardiovascular death) at 12 months (hazard ratio [HR]: 2.52; 95% CI: 1.34 to 4.71; $p = 0.004$) (53). In a prospective study of patients with end-stage renal failure on hemodialysis, impaired endogenous fibrinolysis was detected in 42% of patients using the Global Thrombosis Test and was strongly associated with development of MACEs (HR: 4.25; 95% CI: 1.58 to 11.46; $p = 0.004$), driven by nonfatal MI and stroke (HR: 14.28; 95% CI: 1.86 to 109.90; $p = 0.01$) (46).

The largest study that assessed the usefulness of fibrinolysis assessment using a global assay for cardiovascular risk prediction was a substudy of the PLATO (Ticagrelor versus Clopidogrel in Patients with Acute Coronary Syndromes) trial (55). In this multicenter study of 4,354 patients with ACS, endogenous fibrinolysis was assessed an average of 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 to 1.42) and a 21% relative increase in all-cause death (HR: 1.2; 95% CI 1.03 to 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 to 1.42; $p = 0.042$) but not for turbidity.

The recent RISK-PPCI study assessed 496 patients with STEMI upon arrival, using the point-of-care Global Thrombosis Test (54). Impaired endogenous fibrinolysis, detected in 14% of patients, was highly predictive of recurrent MACEs (HR: 9.1; 95% CI: 5.29 to 15.75; $p < 0.001$), driven by cardiovascular death (HR: 18.5; 95% CI: 7.69 to 44.31; $p < 0.001$) and MI (HR: 6.2; 95% CI: 2.64 to 14.73; $p < 0.001$), particularly within the first 30 days. Cardiovascular risk increased inversely with effectiveness of fibrinolysis. Fibrinolysis remained strongly predictive of MACEs after adjustment for conventional risk factors (HR: 8.03; 95% CI: 4.28 to 15.03; $p < 0.001$).

However, global tests, particularly those that use flow, do not reflect changes that occur in the microenvironment downstream, including the formation of microvascular thrombi that contribute to ischemic 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 defense, because unlike circulating plasmin, t-PA, and u-PA, which 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 (56,57). Animal studies indicate that α2-antiplasmin (a2AP) impairs dissolution of thrombus and mediates microvascular thrombosis, and use of a2AP-inactivating antibody diminishes microvascular thrombosis (57).

Detection of hyperfibrinolysis to assess bleeding. Massively enhanced fibrinolysis due to overexpression of fibrinolytic proteins secondary to trauma, liver failure, or surgery (e.g., liver transplantation or cardiopulmonary bypass) increases bleeding. Hyperfibrinolysis is best detected with the euglobulin lysis time or viscoelastic assays (TEG or ROTEM) (40), although a Cochrane review raised concerns about the accuracy of these assays for bleeding prediction (43).

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

POTENTIAL PHARMACOLOGICAL MODULATION OF FIBRINOLYSIS

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

DRUGS THAT DIRECTLY TARGET PLATELET ACTIVATION OR COAGULATION. Aspirin. In vitro studies have

shown that aspirin renders the fibrin networks looser and prolongs CLT (58). 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 (59).

$P2Y_{12}$ 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 (60,61). In clinical studies, the PLATO substudy used plasma clot lysis (55) and the RISK-PPCI study used the Global Thrombosis Test (54); both showed that endogenous fibrinolytic status was unaffected by dual antiplatelet therapy (DAPT), regardless of whether clopidogrel or ticagrelor was used.

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 caused thrombus instability in vitro, but only cangrelor significantly enhanced endogenous fibrinolysis, although a trend was seen for ticagrelor (62). In vitro, cangrelor alone had no effect on PAI-1 release, but in combination with aspirin, it significantly reduced PAI-1 release from platelets in response to collagen (63).

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 in vitro (64), increasing clot permeability and susceptibility to fibrinolysis (65). Perfusion of human blood containing abciximab, eptifibatide, or tirofiban over freshly-formed thrombus in vitro caused thrombus dissolution through fragmentation (65), an effect that might be mediated through dissociation of fibrinogen from the platelet surface.

Direct oral anticoagulants. The addition of direct oral anticoagulants (DOACs) to human plasma accelerated turbidimetric plasma clot lysis in response to t-PA (66,67) and was concentration-dependent (68). Plasma clot lysis in vitro is enhanced by all DOACs through likely TAFI-mediated and -independent mechanisms (66,69). Clot lysis, measured by a microplate assay, was most rapid in apixaban- and dabigatran-treated patients and slowest in rivaroxaban-treated patients with atrial fibrillation (AF) (70). DOACs enhanced the susceptibility of human in vitro plasma clots to thrombolytic therapy (71,72). Using the Global Thrombosis Test in patients with AF, apixaban, rivaroxaban, and dabigatran all exhibited a trend toward enhancing endogenous fibrinolysis under high-shear conditions in vitro (73). In subjects with AF, endogenous fibrinolysis time assessed using the Global Thrombosis Test was shorter in patients who took apixaban compared with that in patients who took warfarin or aspirin (74). A prospective study of patients with nonvalvular AF assessed before and during apixaban treatment using the Global Thrombosis Test showed that apixaban significantly improved endogenous fibrinolysis, with a maximal effect in those with impaired fibrinolysis pre-treatment (74). A pro-fibrinolytic effect could underlie the results of the ATLAS ACS 2-TIMI-51 (75) and COMPASS (76) studies, in which addition of low-dose rivaroxaban to DAPT significantly reduced cardiovascular events, albeit at a cost of increased bleeding.

DRUGS WITH INDIRECT PLEIOTROPIC EFFECTS.

Statins. Statins exert numerous pleiotropic effects that may be mediated through raising high-density lipoprotein and reducing Lp(a), and include inhibition of platelet activation and coagulation (77). Statins also stimulate fibrinolysis through a reduction in tissue factor, thrombin activity, PAI-1, and an increase in t-PA production (77).

PCSK9 inhibitors. There are abundant data that plasma PCSK9 levels are related to enhanced platelet reactivity (78,79) and may regulate coagulation, as evidenced by a correlation with plasma levels of tissue factor and thrombin-antithrombin complex, and an inverse relationship with protein C level (80). PCSK9 inhibitors may contribute to enhanced fibrinolysis through reduction of Lp(a) (80) and by raising high-density lipoprotein, which inhibits platelet–fibrinogen binding and aggregation in response to thrombin, downregulates coagulation, and stimulates fibrinolysis (79).

POTENTIAL FUTURE ANTITHROMBOTIC THERAPIES.

Factor XI inhibition. In animal models, factor XI inhibition enhanced thrombolysis, and treatment with factor XI antisense oligonucleotide significantly attenuated thrombus formation and fibrin deposition with formation of unstable thrombi (81). A subcutaneous factor XI–directed antisense oligonucleotide showed safety in a phase 2 study in humans (81,82).

Factor XII inhibition. Factor XII inhibition reduces clot firmness, and thrombi created in factor XII–deficient mice are unstable and prone to embolization (83). In a carotid injury model, recombinant fully human factor XIIa activity neutralizing antibody (3F7) dose dependently reduced thrombus formation at arterial shear rates, without an increase in bleeding (84).

TAFI inhibition. DS-1040 is a novel compound that inhibits activated TAFI. In animal studies, DS-1040 enhanced endogenous fibrinolysis, and, in a first-in-human phase 1 study, intravenous infusion caused a dose-dependent decrease in both TAFIa activity and in CLT (85).

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 (86) for a meaningful risk marker because: 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; 6) the tests are acceptable to patients; and 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, DOACs show the most promise. This is indirectly supported by clinical studies that show that anticoagulation, in addition to antiplatelet therapy, can reduce cardiovascular risk in stable disease or ACS, albeit at the cost of increased bleeding (75,76). Although this strategy clearly cannot be recommended for all patients with ACS, assessing endogenous fibrinolysis could identify those at highest risk and who would benefit most from anticoagulation. For endogenous fibrinolysis assessment to translate into an improvement in clinical outcome, large prospective trials are now needed to assess whether "personalized" antithrombotic therapy to enhance fibrinolysis in patients with ACS 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 appears to be a novel risk factor that is independent of traditional cardiovascular risk factors and unaffected by DAPT.

The best clinical test to assess endogenous fibrinolysis is unclear. Most data showing the importance of endogenous fibrinolysis as a marker of cardiovascular risk have been obtained using a global assay that uses 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 t-PA. This would assess the contribution of flow and/or shear, adherent neutrophils, and platelets, including through thrombin generation and release of PAI-1, TAFI and a2AP, to both thrombus formation and lysis. Although some 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.

Although 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. In contrast, turbidimetric tests use only plasma, do not take account of other blood constituents or the effects of flow, and use external agonists.

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

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