JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY
VOL. 65, NO. 16, 2015

ISSN 0735-1097/$36.00

http://dx.doi.org/10.1016/j.jacc.2015.02.040

REVIEW TOPIC OF THE WEEK

Endogenous Fibrinolysis

An Important Mediator of Thrombus Formation and Cardiovascular Risk

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ABSTRACT

Most acute cardiovascular events are attributable to arterial thrombosis. Plaque rupture or erosion stimulates platelet activation, aggregation, and thrombosis, whilst simultaneously activating enzymatic processes that mediate endogenous fibrinolysis to physiologically maintain vessel patency. Interplay between these pathways determines clinical outcome. If proaggregatory factors predominate, the thrombus may propagate, leading to vessel occlusion. However, if balanced by a healthy fibrinolytic system, thrombosis may not occur or cause lasting occlusion. Despite abundant evidence for the fibrinolytic system regulating thrombosis, it has been overlooked compared with platelet reactivity, partly due to a lack of techniques to measure it. We evaluate evidence for endogenous fibrinolysis in arterial thrombosis and review techniques to assess it, including biomarkers and global assays, such as thromboelastography and the Global Thrombosis Test. Global assays, simultaneously assessing proaggregatory and fibrinolytic pathways, could play a role in risk stratification and in identifying impaired fibrinolysis as a potential target for pharmacological modulation. (J Am Coll Cardiol 2015;65:1683–99) © 2015 by the American College of Cardiology Foundation. Open access under CC BY-NC-ND license

Cardiovascular disease is the leading cause of morbidity and mortality in developed countries. The common pathological process responsible for the majority of these disorders, including acute coronary syndrome (ACS) and ischemic stroke, is the development of an occlusive arterial thrombus.

Disruption of an atherosclerotic plaque through rupture or erosion creates a prothrombotic environment to circulating platelets and procoagulant factors. The major thrombogenic components contained within the atherosclerotic plaque include tissue factor and collagen (1–3). Exposure to this thrombotic milieu provides a potent stimulus for platelet activation, aggregation, and thrombosis (**Figures 1A and 1B**). Activation of the coagulation cascade also leads to direct activation of the enzymatic processes that mediate endogenous fibrinolysis (**Figure 1C**). This interaction is important to ensure that thrombosis is controlled and vessel patency is maintained.

The interplay between these opposing pathways is likely to determine the occurrence and clinical outcome of a resulting thrombus. If proaggregatory and procoagulant factors predominate, an intraluminal thrombus may propagate and lead to complete vessel occlusion, with subsequent lasting downstream tissue damage (**Figure 1B**). If, in contrast, the prothrombotic factors are balanced by a healthy fibrinolytic system, then a thrombus may not develop or may not cause lasting vessel occlusion (**Figure 1C**).

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Manuscript received January 12, 2015; revised manuscript received February 20, 2015, accepted February 23, 2015.

ABBREVIATIONS AND ACRONYMS

ACS = acute coronary syndrome(s)

AMI = acute myocardial infarction

GTT = Global Thrombosis Test

MACE = major adverse cardiovascular event(s)

PAI = plasminogen activator inhibitor

ROTEM = rotational thromboelastometry

SR = spontaneous reperfusion

STEMI = ST-segment elevation myocardial infarction

TAFI = thrombin-activatable fibrinolysis inhibitor

TEG = thromboelastography

t-PA = tissue-type plasminogen activator

IMPORTANCE OF THE ENDOGENOUS FIBRINOLYTIC SYSTEM IN ACS

An intact endogenous fibrinolytic system serves to actively prevent the buildup of formed thrombi through dissolution of an arterial thrombus (**Central Illustration**). Despite a wealth of evidence supporting its role in preventing lasting arterial occlusion, this pathway has been relatively overlooked as compared with the understanding, monitoring, and pharmacological modulation of platelet reactivity. This may have occurred due to limitations of earlier methods to robustly measure the activity of the fibrinolytic system. Additionally, besides the use of plasminogen activators to achieve acute thrombolysis in the setting of acute myocardial infarction (AMI) and stroke, pharmacological options available to manipulate the fibrinolytic state have been very limited.

Evidence from clinical, histopathologic, and autopsy studies (4–9), as well as clinical observations, support the proposal that AMI may represent a failure of timely, spontaneous endogenous thrombolysis. In 585 patients presenting with ST-segment elevation myocardial infarction (STEMI), spontaneous reperfusion (SR), evidenced by electrocardiographic resolution of ST-segment changes, was observed in 14.9%, and normal coronary flow on angiography was observed in 14.7% of patients (10). In 1,667 patients assigned to the primary percutaneous coronary intervention arm of the ASSENT 4 (Assessment of the Safety and Efficacy of a New Treatment Strategy for Acute Myocardial Infarction) trial (11), SR was associated with a lower composite of death, heart failure, or shock compared with those with persistent ST-segment elevation. In 710 STEMI patients undergoing primary percutaneous coronary intervention, SR was observed in 22%, and these patients had a lower incidence of death, congestive heart failure, and recurrent ACS at 30 days than those without SR (12). Furthermore, histopathologic studies evaluating aspirated coronary thrombi from patients with STEMI have demonstrated significant heterogeneity in the composition and age of the culprit thrombi (4–7). Among 1,362 STEMI patients, up to 40% demonstrated lytic or organized thrombi, signifying that thrombus formation occurred days to weeks before final vessel occlusion (7). This underpins the notion that thrombus generation is an active and dynamic process, where constant thrombosis and thrombolysis may occur in concert.

Autopsy studies of healed plaque disruptions also provide evidence of thrombus formation as a dynamic process (8,13). Plaque instability appears to be present for some time before an occlusive thrombus is formed, and may be asymptomatic. Nonocclusive mural thrombi may form over plaque disruptions, leading to phasic progression of atherosclerotic lesions, but without presenting as ACS (13,14).

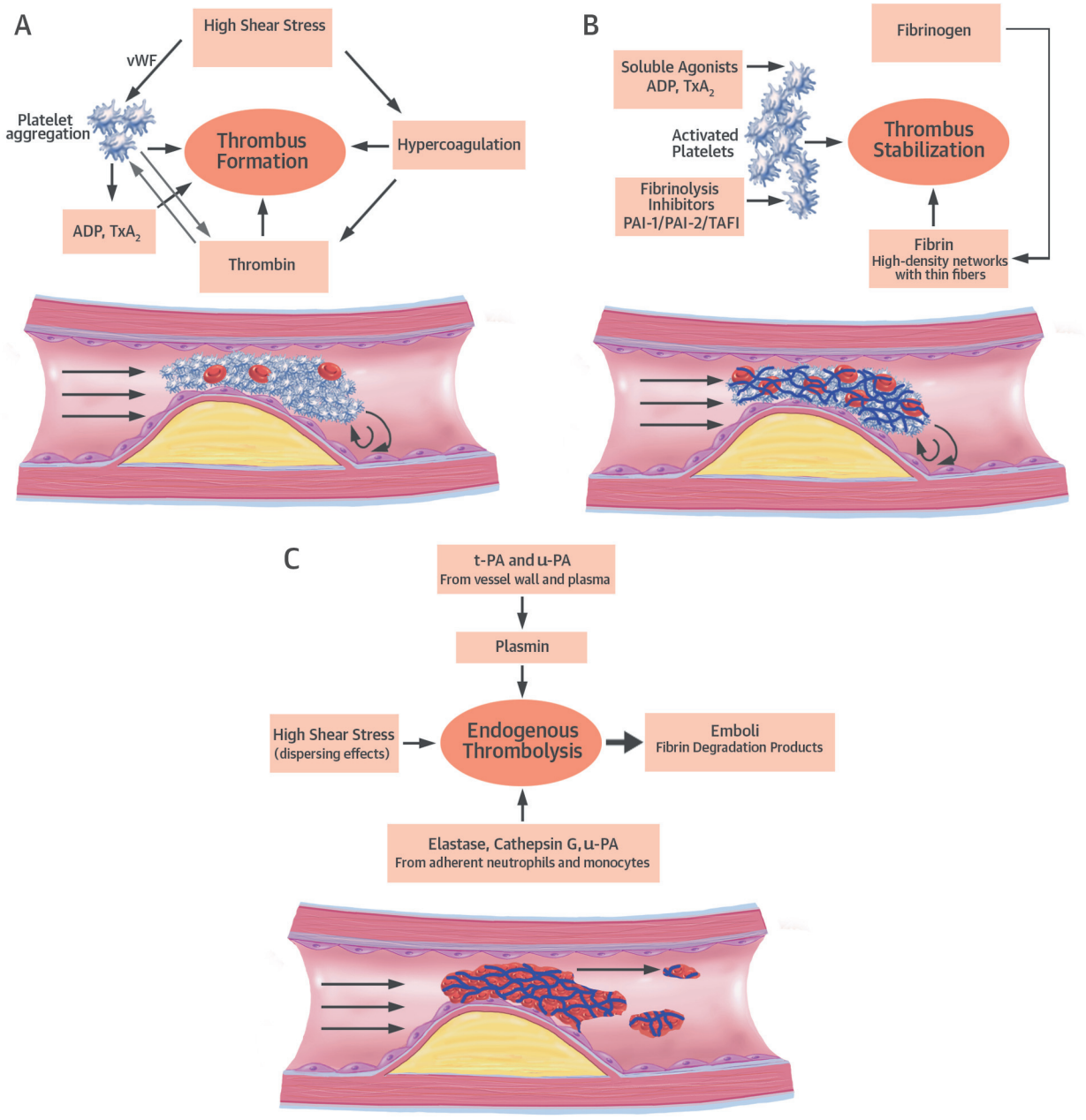
Despite the fact that plaque rupture represents a common unifying event for coronary thrombosis, there is significant variability in clinical manifestation and outcome. This variability may be explained, in part, by the role of endogenous fibrinolysis in limiting the propagation of formed thrombi and preventing total coronary occlusion (**Central Illustration**). In this paper, we review the methods currently available to assess endogenous fibrinolysis and evaluate the evidence for the role of endogenous fibrinolysis as a mediator of arterial thrombus formation in coronary disease.

FACTORS DETERMINING RESISTANCE OF THROMBUS TO LYSIS

Whole blood clots are more resistant to lysis than plasma clots, implying that blood cells and fibrin are responsible for the resistance (15) (**Central Illustration**). Platelets play the main role in resistance, but red cell–derived microparticles can also contribute to thrombin generation, whereas elastase released from leukocytes trapped or adherent to the thrombus exerts a plasmin-independent fibrinolytic effect. Arterial (platelet-rich) thrombi are much more resistant to lysis than erythrocyte-rich venous thrombi (16). The mechanisms through which platelets contribute to thrombolysis resistance are 3-fold (**Central Illustration**):

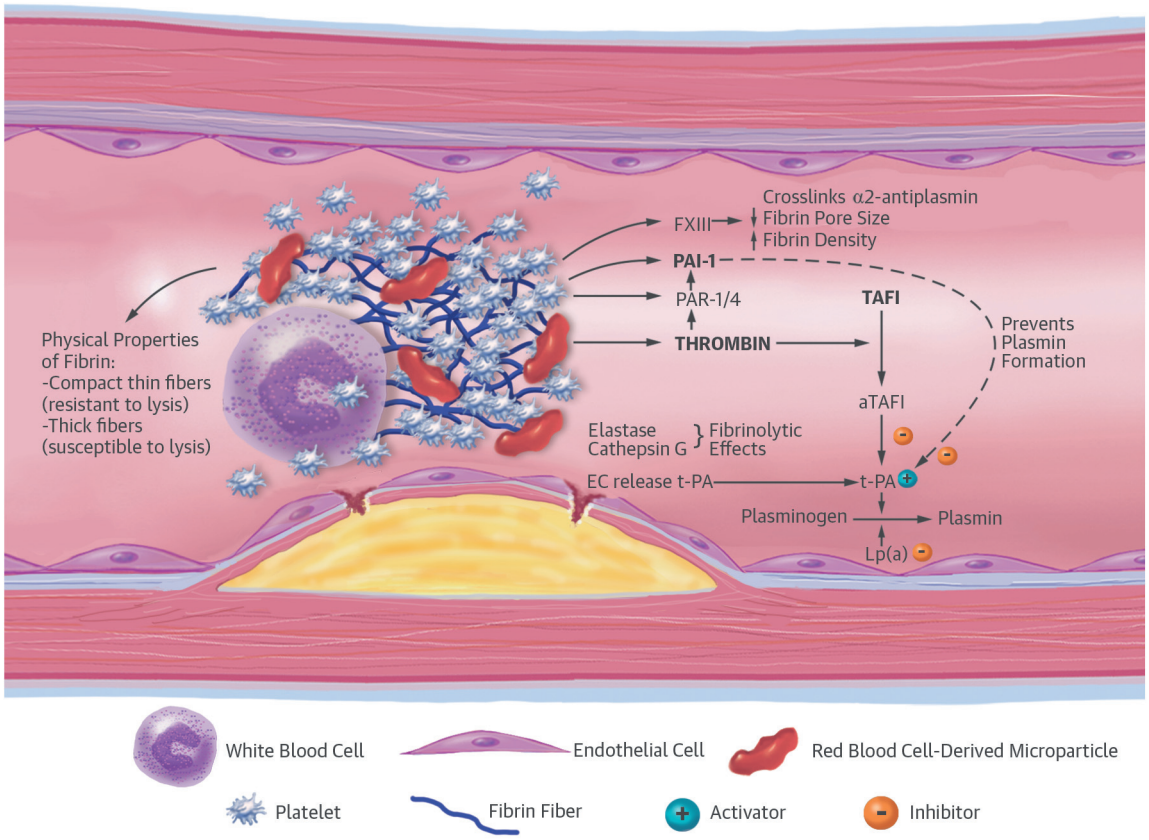
1. Platelets contain >90% of the circulating plasminogen activator inhibitor (PAI)-1. During aggregation, in response to thrombin, PAI-1 is released from platelets into the thrombus mass and is the major determinant of arterial thrombolysis resistance (17).
2. The procoagulant activity or contribution of platelets to thrombin generation is extremely important, not only in the generation of, but also in the lysis of the formed thrombus. A high shear stress milieu, such as that found in an artery with a severe stenosis, will trigger microparticle release from activated platelets, resulting in a burst of thrombin generation. In addition to PAI-1, thrombin-activatable fibrinolysis inhibitor (TAFI) also contributes to thrombolysis resistance.

FIGURE 1 **The Mechanism and Importance of Endogenous Fibrinolysis in Regulation of Occlusive Arterial Thrombus Formation, and its Relevance to Laboratory Tests Assessing Thrombotic Risk**



(A) Under conditions of high shear, such as those that exist in a narrowed coronary artery, stimulation of platelet aggregation by von Willebrand Factor (vWF) results in the formation of thrombin, the key mediator of thrombus formation. **(B)** The thrombus achieves structural stability and resistance to dislodgement and to thrombolysis through fibrin (which crosslinks cells to provide structural stability), plasminogen activator inhibitor (PAI)-1 released from activated platelets, and activation of thrombin-activatable fibrinolysis inhibitor (TAFI) by thrombin. **(C)** Endogenous thrombolysis: physiological processes that exist to prevent lasting occlusive thrombus formation, including the release of tissue plasminogen activator (t-PA) and urokinase plasminogen activator (u-PA) from the vessel wall and plasma, the release of elastase and cathepsin from adherent neutrophils and monocytes, and the dispersing effect of flow. ADP = adenosine diphosphate; TxA_2 = thromboxane A_2.

CENTRAL ILLUSTRATION **Endogenous Thrombolysis in CV Disease: Determinants of Spontaneous Thrombolysis Under Arterial Flow Conditions**



Okafor, O.N. et al. J Am Coll Cardiol. 2015; 65(16):1683–99.

Thrombin converts plasminogen to plasmin, which breaks down the cross-linked fibrin into soluble fibrin degradation products. t-PA is mainly responsible for the dissolution of fibrin formed in the circulation. Inhibitors of thrombolysis include the release of PAI-1 from platelets, secretion of active PAI-1 from aggregated platelets, and clot retraction. Potentiators of thrombolysis include: the release of elastase and cathepsin G from white blood cells that become trapped in the thrombus, which directly break down fibrin; the plasminogen activators uPA and t-PA (released from endothelial cells); and fibrin structural properties. The thickness and porosity of fibrin fibers will also determine structural stability and susceptibility to thrombolysis. Lp(a), a homologue of plasminogen, can inhibit t-PA–mediated plasminogen activation. aTAFI = activated thrombin-activatable fibrinolysis inhibitor; CV = cardiovascular; EC = endothelial cell; FXIII = coagulation factor XIII; Lp(a) = lipoprotein (a); PAI-1 = plasminogen activator inhibitor; PAR = protease-activated thrombin receptor; TAFI = thrombin-activatable fibrinolysis inhibitor; t-PA = tissue plasminogen activator.

3. The structure and stability of the resultant fibrin network is also a determinant of thromboresistance (18). Platelets play an important role in regulating fibrin network structure (19). Coagulation factor XIII (FXIII), in addition to cross-linking fibrin, also plays an important role in thrombolysis resistance. Platelets are abundant in FXIII, and upon activation, FXIII-A exposure on the surface membrane exerts an antifibrinolytic function by cross-linking the major plasmin inhibitor α2-antiplasmin to fibrin, thus inhibiting plasmin-mediated clot degradation (20,21). FXIII alters the structure of the fibrin network to reduce pore size and increase fiber density, thus increasing clot stability and resistance to lysis (22). Thrombi formed in FXIII-deficient blood lyse more quickly than in normal blood, and FXIII concentrate normalized lysis (23). A common genetic polymorphism of FXIII has been shown to increase the risk of myocardial infarction (24).

MEASUREMENT OF FIBRINOLYTIC STATUS

Interest in the endogenous fibrinolytic system has fueled the development of techniques to assess and quantify the activity of this important pathway. Many

of the early studies utilizing these techniques provided compelling evidence of endogenous fibrinolysis in arterial thrombogenesis, although no current test has been adopted into widespread clinical use.

Current techniques include: 1) the measurement of 1 or several protein components of the fibrinolytic cascade; or 2) global assessment of fibrinolytic capacity utilizing techniques such as euglobulin clot lysis time, thromboelastography (TEG) (Haemoscope Corporation, Niles, Illinois) or rotational thromboelastometry (ROTEM) (Tem International GmbH, Munich, Germany), and the most recent Global Thrombosis Test (GTT) (Thromboquest Ltd., London, United Kingdom). These techniques are described in more detail in the following text.

PLASMA MARKERS OF FIBRINOLYSIS

The fibrinolytic system shares several similarities with the coagulation cascade, involving a series of proteolytic enzymatic steps that culminate in the conversion of plasminogen to plasmin to achieve fibrin dissolution (**Figure 1C**, **Central Illustration**). Thrombin converts plasminogen to plasmin, which breaks down the cross-linked fibrin into soluble fibrin degradation products. Tissue-type plasminogen activator (t-PA) is mainly responsible for the dissolution of fibrin formed in the circulation. Fibrinolysis can be inhibited either by antagonizing plasmin through alpha 2-antiplasmin or by PAIs. PAI-1, stored in the alpha granules of platelets, is mainly responsible for resistance to fibrinolysis. Activation of protease-activated thrombin receptor 1 by thrombin results in synthesis and secretion of active PAI-1 from aggregated platelets and thrombin activation of TAFI, which inhibits the t-PA-mediated conversion of plasminogen to plasmin. Plasma lipoprotein (a) [Lp(a)], a homolog of plasminogen, can inhibit t-PA-mediated plasminogen activation.

Measurement of individual proteins involved in fibrinolysis, as a surrogate marker of overall fibrinolytic activity, can be undertaken using immunoassays. Studies have focused on the measurement of a number of biomarkers of fibrinolysis, including t-PA (25-30), PAI-1 (31), alpha-2 antiplasmin, alpha2-antiplasmin-plasmin complex (31), markers of fibrin degradation products (D-dimer and soluble fibrin) (25,28,31), and, more recently, TAFI (31) and Lp(a) (31,32).

Given the potential role of the fibrinolytic system in the pathogenesis of ACS, studies have attempted to elucidate the relationship between plasma biomarkers and incipient cardiovascular risk. The main limitations of this approach are knowing the relative importance and contribution of any biomarker to the overall fibrinolytic system at any given point, knowing whether to measure levels or activity of biomarkers, and the confounding association between fibrinolytic markers and more established cardiovascular risk factors (33,34). Overall, the outcome of studies evaluating the role of plasma markers of fibrinolysis as independent predictors of cardiovascular risk has been disappointing, with much conflicting evidence and a number of positive studies only demonstrating a weak association (31). This, combined with methodological problems with the assays, has resulted in greater emphasis being placed on more global assays of fibrinolytic activity.

EUGLOBULIN CLOT LYSIS TIME

The euglobulin fraction (containing the key activators of the fibrinolytic cascade, including plasminogen activators, plasminogen, and plasmin) is precipitated from citrated plasma and calcium is added to promote clot formation. The time taken to lyse this clot is utilized as a measure of fibrinolytic activity (35,36). This technique from the 1950s has now been superseded by more rapid, physiological tests of fibrinolytic capacity.

THROMBOELASTOGRAPHY

TEG is a global test of coagulation status, simultaneously assessing clot development, stabilization, and dissolution. Another related and commercially-available technique is ROTEM (37,38). TEG utilizes a pin suspended by a torsion wire into a cylinder to measure the physical properties of a clot. The torsion wire is connected to a mechanical-electrical transducer and relays information on the speed and

TABLE 1 Comparison of Thromboelastography (ROTEM) and the GTT, With Respect to Assessment of Thrombosis

Measurement	ROTEM	GTT
Thrombus	Cross-linked fibrin clot	Platelet-rich fibrin clot
Relevance	Venous thrombosis	Arterial thrombosis
Flow (shear rates/s)	Static (0.1/s)	High shear (>10,000/s)
Blood sample	Citrate-anticoagulated	Native blood
Thrombus resistance	No	Yes
PAI-1 involvement in lysis	No	Yes
Platelets procoagulant effect	Little	Significant
Activator	Tissue factor/kaolin (extrinsic and intrinsic coagulation pathways)	High shear stress only
Hyperfibrinolysis (t-PA)	Yes	Yes
Hypofibrinolytic state	No	Yes

GTT = global thrombosis test; PAI-1 = plasminogen activator inhibitor-1; ROTEM = rotational thromboelastometry; t-PA= tissue-type plasminogen activator.

TABLE 2 Studies From 1999 to 2015 That Utilized Multivariate Analysis in Patient Cohorts >1,000 to Assess the Predictive Value of Plasma Fibrinolysis Markers for Adverse CV Events

First Author (Ref. #)	Design	Patients (n)	Population	Follow-Up	Endpoint	Results
Kinlay et al. (28)	RCT	2,860	ACS patients enrolled in MIRACL study	16 weeks	CV events (death, nonfatal MI, cardiac arrest, or worsening angina)	t-PA antigen associated with increased risk of CV events (HR: 1.25; p = 0.0014). This correlation was attenuated following adjustment for risk factors (HR: 1.14; p = 0.08)
Zamani et al. (106)	RCT	2,925	ACS patients enrolled in MIRACL study	16 weeks	Death and recurrent nonfatal ACS (MI or unstable angina)	t-PA antigen associated with primary endpoints, but after adjustment for risk factors, HR fell from 1.90 to 1.27 for death (p = NS), and HR 1.20 for ACS (p = NS).
Wang et al. (66)	Prospective	3,209	Subjects in 6th cycle of Framingham Offspring Study (1995–1998)	10 yrs	Death and major CV events (MI, unstable angina, heart failure, and stroke) and nonmajor CV events	Multiple biomarkers, including D-dimer and PAI-1. Following multivariate adjustment, HR for D-dimer associated with death was 1.24; 95% CI: 1.02–1.50; p = 0.03; and HR: 1.24 for PAI-1 in relation to CV events (p = 0.03).
Folsom et al. (34)	Prospective	6,391	Subjects without atherosclerosis	4.6 yrs	Cancer death, mortality, and CAD (MI or coronary death), and CV disease (cardiac arrest, angina ± revascularization and stroke)	D-dimer, factor VIIIc and PAP not predictive of CV disease, but independently associated with cancer death and total mortality. Following adjustment for risk factors, mortality increased for each quartile increment in D-dimer (33% increase; 95% CI: 15–54), factor VIIIc (26% increase; 95% CI: 11–44), and PAP (20% increase; 95% CI: 4–38) (p values not published).
May et al. (25)	Prospective	3,582	Women without prior CAD	4.7 yrs	Development of CV death, MI, or coronary revascularization	D-dimer, t-PA antigen, and vWF were not associated with development of CAD after adjustment for CV risk factors.
Cushman et al. (108)	Nested case-control study	5,201 (146 selected cases)	Patients without baseline vascular disease	2.4 yrs	Coronary death, MI, and angina.	D-dimer and PAP levels, but not PAI-1, predicted MI or coronary death, but not angina. D-dimer values above median associated with RR: 2.5; 95% CI: 1.1–5.9; and for PAP with RR: 3.1; 95% CI: 1.3–7.7, independent of other risk factors.
Nordenhem et al. (107)	Case-control study	1,267	Patients with first MI identified	Matched to control group	MI	Plasma t-PA/PAI-1 complex associated with MI, with synergistic interaction in male smokers (OR: 4.6; 95% CI: 3.3–6.5) or diabetics (OR: 7.9; 95% CI: 3.9–16.1) (p values not published).
Smith et al. (64)	Prospective	2,398	Men age 49–65 yrs	4 yrs	CV events (coronary heart disease and ischemic stroke combined)	After adjusting for risk factors, fibrinogen (HR: 1.26; p = 0.005), D-dimer (HR: 1.34; p = 0.001) and PAI-1 (HR: 1.24; p = 0.013) were independent risk factors for CV events. Factor VIIc was inversely related to CV events (HR: 0.75; p = 0.001).

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TABLE 2 Continued

First Author (Ref. #)	Design	Patients (n)	Population	Follow-Up	Endpoint	Results
Morange et al. (109)	Prospective	1,057	Patients with CAD (AtheroGene Study)	6.6 yrs	CV death and nonfatal CV events (MI and stroke)	vWF, fibrinogen, TAT, D-dimers, and PAP were all associated with CV death but not with nonfatal CV events. After adjustment for risk factors and CRP, only fibrinogen and D-dimer remained associated with CV death (HR: 1.27; 95% CI: 1.04–1.55; p = 0.019).
Tregouet et al. (110)	Prospective	1,668	Patients with CAD (AtheroGene Study)	2.3 years	CV death and nonfatal CV event (MI)	Activated TAFI independently associated with risk of CV death (HR: 2.38; 95% CI: 1.56–3.63; p < 0.0001), even after adjustment for risk factors (HR: 1.69; 95% CI: 1.07–2.67; p = 0.01). Total TAFI not associated with CV events.
Gaw et al. (111)	Prospective	5,732	Elderly patients with risk factors for, or established vascular disease	3.2 yrs	CV death, nonfatal MI, fatal or nonfatal stroke	Lp(a) levels not associated with primary endpoint (HR: 1.05; 95% CI: 1.00–1.11; p = NS).
Bennet et al. (112)	Case-control study	2,047	Patients without CAD or stroke at baseline who experienced an MI or coronary death	NA	First-ever MI or coronary death	Lp(a) values in the top vs. bottom third, after multivariate adjustment for CV risk factors, OR: 1.60; 95% CI: 1.38–1.85 (p values not published). Progressive increase in OR with higher Lp(a) levels.
Willeit et al. (91)	Prospective case-control study	1,925	Patients without CV disease who experienced MI or coronary death	19.4 yrs	First-ever MI or coronary death	OR for D-dimer was 1.08 (p = 0.019), OR for t-PA antigen 1.05 (p = 0.167). and OR for Lp(a) 1.24 (p < 0.001).
Wannamethee et al. (92)	Prospective study	3,217	Men age 60–79 yrs without CAD	7 yrs	Coronary death, nonfatal MI, and uncomplicated angina	After adjustment for risk factors, D-dimer associated with MI/coronary death (HR: 1.18; p = 0.02), but not with angina (HR: 0.93; p = NS).
Chien et al. (93)	Prospective cohort study	3,484	Chinese patients without CAD	13.8 yrs	All-cause death, stroke, and CAD	Lp(a) levels did not correlate with risk of CV disease (HR: 0.81; p = NS).
Gurdasani et al. (94)	Prospective cohort study	18,720	Healthy subjects, age 39–79 yrs	11.4 yrs	Peripheral artery disease, stroke, and CAD-related events	Lp(a) levels associated with CAD hospitalization and mortality (HR: 1.13; p < 0.00001).
Nestel et al. (95)	Prospective study	7,863	Patients with prior coronary event	6 yrs	Coronary death, nonfatal MI, ischemic stroke, revascularization, total CV events, and total coronary events	Lp(a) levels correlated with CV events (p < 0.001), total CV events (HR: 1.23; p = 0.002), and coronary events (p = 0.03).
Kwon et al. (96)	Prospective	1,494	Type 2 diabetic patients with CAD	4.4 yrs	MACE (cardiac deaths and nonfatal MI)	Highest Lp(a) level tertile associated with MACE (HR: 2.89; p = 0.005).
Kwon et al. (97)	Prospective	6,252	Patients with suspected CAD	3.1 yrs	MACE (cardiac death and nonfatal MI)	Elevated Lp(a) associated with MACE (HR: 1.773; p = 0.005).
Canoui-Poitrine et al. (100)	Prospective cohort study	9,711	Men age 50–59 yrs free of CAD and stroke	10 yrs	CAD events (angina, MI, and coronary death) and ischemic stroke	Lp(a) levels associated with CV events (HR: ~1.2; p = 0.001) after adjustment for risk factors.
Virani et al. (101)	Prospective	13,318 (n = 3,467 blacks, n = 9,851 Caucasians)	African-American and Caucasian adults without CHD or stroke	20 yrs	CV events (coronary death, MI, silent MI, revascularization) and stroke	Lp(a) levels associated with CV events. Quintile analysis for the highest compared with the lowest quintile demonstrated an HR: 1.35 (p = 0004) for blacks and HR: 1.27 (p = 0.001) for whites.
O'Donoghue et al. (102)	Prospective	6,708	Patients with CAD from 3 studies (PEACE, CARE, and PROVE-IT-TIMI 22 trial)		MACE (composite of CV death, MI, or stroke)	No association between Lp(a) levels and MACE in any of the 3 trials individually or combined.

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TABLE 2 Continued

First Author (Ref. #)	Design	Patients (n)	Population	Follow-Up	Endpoint	Results
Suk Danik et al. (103)	Prospective	27,791	Healthy women age >45 yrs	10 yrs	MACE (nonfatal MI, nonfatal cerebrovascular event, coronary revascularization, or CV death)	Lp(a) levels in the highest vs. lowest quartiles associated with adverse events (HR: 1.35; $p < 0.001$ for trend across quartiles).
Kamstrup et al. (104)	Prospective	9,330	Subjects without prior CAD	10 yrs	CAD (including MI) or death	Raised Lp(a) level associated with HR: 1.09 (95% CI: 1.06–1.12; $p = 0.93$) for MI and 1.06 (95% CI: 1.04–1.08; $p = 0.86$) for CAD.
Shilpak et al. (105)	RCT	2,763	Post-menopausal women age <80 yrs with CAD	4.1 yrs	CV events (nonfatal MI and CV death)	Lp(a) levels associated with CV events (HR: 1.54; 95% CI: 0.99–2.39; $p = 0.03$)

ACS = acute coronary syndromes; CAD = coronary artery disease; CARE = Cholesterol And Recurrent Events; CHD = coronary heart disease; CI = confidence interval; CRP = C-reactive protein; CV = cardiovascular; HR = hazard ratio; Lp(a) = lipoprotein (a); MACE = major adverse cardiovascular events; MI = myocardial infarction; MIRACL = Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering; NA = not applicable; NS = not significant; OR = odds ratio; PAP = plasmin-alpha2-antiplasmin complex; PEACE = Prevention of Events With Angiotensin-Converting Enzyme Inhibitor Therapy; PROVE-IT-TIMI 22 = Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis In Myocardial Infarction 22; RCT = randomized controlled trial; RR = relative risk; TAFI = thrombin-activatable fibrinolysis inhibitor; TAT = thrombin-antithrombin complex; vWF = von Willebrand factor; other abbreviations as in Table 1.

dynamics of clot formation. As blood clot formation occurs around the pin, fibrin strands form between the cylindrical cup and pin. With additional rotation of the cylindrical cup, this is transmitted to the pin and the resulting mechanical-electrical transduction is depicted in a numerical and graphical representation. With the ROTEM technique, movement is instead generated from the oscillation of the pin/wire transduction system, while the cup is held immobile and an optical detection system is utilized to transduce the signal. TEG can be modified to use a variety of different activators and inhibitors to provide information on specific components of the coagulation system, including platelet function testing and fibrinolytic status (38-45). In fibrinolysis assessment, TEG is typically compared in the presence and absence of the fibrinolysis inhibitor aprotinin (37,46). **Table 1** shows the features of thromboelastography and how these compare with the GTT.

There are a number of important limitations to the use of TEG as a clinical tool to assess global thrombotic status. TEG was originally designed for native, nonanticoagulated blood (47), but subsequent modifications have included the use of activators of coagulation and additional reagents to evaluate specific components of hemostasis (38,47). This has helped standardize the initiation of coagulation, but does not reflect a patient's physiological state. Although TEG is a useful tool for assessing bleeding risk, for example in cardiac surgery, its practical value in assessing the (spontaneous) thrombolytic status of patients or the effect of medications is questionable. The shortcomings of this technique begin with the testing of citrated and recalcified blood. The effect of extracellular calcium concentration on coagulation indexes and thromboelastography results is significant (48). There are significant differences in TEG results between fresh native whole blood and recalcified citrated whole blood (49,50) and the correlation between TEG results performed on kaolin- versus nonkaolin-activated native and citrated blood is poor (51).

In the absence of shear or any other platelet-activating stimuli, clot formation can be initiated either by intrinsic (kaolin, ellagic acid) or extrinsic (tissue factor) activators, and the test results vary accordingly. However, the major limitation of TEG is that it fails to assess the procoagulant (thrombin-generating) and fibrinolysis-inhibiting (PAI-1; TAFI) properties of platelets. Furthermore, the use of gentle rotation of a cylindrical cup more closely resembles the low shear stress environment encountered with venous stasis and does not reflect the high-shear situation in stenosed arteries. Furthermore, this mitigates the contribution of platelet activation and

subsequent thrombin generation, which play key roles in arterial thrombogenesis.

TEG results have only demonstrated a weak correlation with standard tests of coagulation (52), with no formal validation or standardization (53) and significant interlaboratory variability; coefficients of variation range between 8% and 40% for TEG and up to 4% to 84% for ROTEM (54). Due to the problems with standardization and determination of normal reference values, TEG is better utilized as a measure of the change in coagulation status over time, when a patient's baseline results are already known (55). Despite these limitations, TEG benefits from its availability as a point-of-care test, providing rapid information on the coagulation profile of patients.

GLOBAL THROMBOSIS TEST

The GTT is a newer point-of-care test that simultaneously assesses platelet reactivity, thrombosis, and thrombolytic activity, from a single, nonanticoagulated blood sample (56,57). This technique utilizes native, nonanticoagulated blood that is free of any external agonists (Table 1). Platelets become activated by the high shear stress generated by the passage of blood through a conical tube containing narrow gaps. The predominant stimulus for platelet activation in severely-narrowed atherosclerotic coronary arteries is pathologically high shear stress (>10,000 s^{-1}), which leads to rapid platelet activation. The GTT mimics this pathological environment to provide high shear stress as the primary stimulus for platelet aggregation, platelet microparticle, and thrombin generation, resulting in occlusive thrombus formation (58,59). The time taken for an occlusive thrombus to form in the space downstream, reflecting platelet aggregation and initiation of coagulation, is manifested in the arrest of flow as detected by an optical sensor, and is termed the occlusion time (OT, s). The restart of blood flow, due to spontaneous dissolution of the formed thrombus, represents endogenous thrombolytic activity and is recorded again by an optical sensor and termed the lysis time (LT, s).

TABLE 3 Clinical Studies Evaluating the GTT in the Prediction of Cardiovascular Risk

First Author (Ref. #)	Population	Patients (n)	Follow-Up	Methods	Primary Endpoint	Results
Sharma et al. (60)	End-stage renal disease patients on hemodialysis	216	276 ± 166 days	GTT	MACE (CV death, nonfatal MI, CVA, and peripheral arterial thrombosis)	Impaired endogenous thrombolysis (LT >3,000 s) strongly associated with MACE (HR: 4.25; p = 0.004), nonfatal MI, and CVA (HR: 14.28; p = 0.0 = 1) and peripheral thrombosis (HR: 9.08; p = 0.003)
Saraf et al. (61)	ACS patients receiving dual antiplatelet therapy	300	12 months	GTT	MACE (CV death, nonfatal MI, or CVA)	LT >3,000 s was an independent predictor of MACE (HR: 2.52; p = 0.004) and CV death (HR: 4.2; p = 0.033).
Saraf et al. (61)	ACS vs. healthy control subjects	300	N/A	GTT	MACE (CV death, nonfatal MI, or CVA)	OT prolonged in ACS (428 s vs. 378 s; p < 0.001) and LT shorter in ACS (1,053 s vs. 1,362 s; p < 0.001) than in control subjects
Suehiro et al. (75)	Healthy subjects of smoking and nonsmoking status	Smokers = 76 vs. nonsmokers = 63	3 months	GTT	Effect of smoking on thrombotic profile	LT was significantly longer in smokers than in nonsmokers (1,794 s vs.1,530 s; p = 0.029) with no significant difference in OT
Ikarugi et al. (76)	Healthy young males and elderly males	Young = 30 vs. elderly = 34	N/A	GTT	Effect of age, smoking, and exercise on thrombotic profile	LT was significantly longer in elderly vs. young (p < 0001), and prolonged in elderly smokers than nonsmokers (p < 0.001)
Suehiro et al. (77)	Males with MetS vs. control subjects	MetS = 30 vs. control = 53	N/A	GTT	Comparison of thrombotic profile between groups	LT significantly longer in MetS than in control subjects (1,494 s vs. 1,246 s). PAI-1 level correlated with LT (p < 0.01)
Rosser et al. (98)	ACS or stable coronary disease randomized to vorapaxar vs. placebo, in addition to standard of care	57	N/A	GTT	Thrombotic status, as shown by OT and LT of GTT	Vorapaxar treatment prolonged OT (561 s vs. 372 s; p = 0.003) and shortened LT (1,158 s vs. 1,733 s; p = 0.016)
Taomoto et al. (99)	Acute cerebrovascular disease (CVA) vs. healthy control subjects	CVA = 185 control subjects = 195	N/A	GTT	Thrombotic status, as shown by OT and LT of GTT	In stroke patients, OT was shorter (p < 0.0001) and LT was longer (p < 0.0001) than in healthy control subjects

CVA = cerebrovascular accident (stroke); LT = lysis time; MetS = metabolic syndrome; OT = occlusion time; other abbreviations as in Tables 1 and 2.

TABLE 4 Studies Utilizing TEG in the Evaluation of Platelet Reactivity and its Correlation to the Risk of Ischemic Events in Nonsurgical Cardiovascular Patients

First Author (Ref. #)	Population	Patients (n)	Follow-Up	Method	Primary Endpoint	Results
Jeong et al. (113)	PCI-treated patients receiving aspirin and clopidogrel	197	24 months	MA-thrombin TEG measurements, conventional aggregometry, and genotyping	Relationship between MA-thrombin on high on-treatment platelet reactivity (HPR) and long-term MACE	HPR and high MA-thrombin were both independently associated with MACE (HR: 3.09 and 2.24, respectively). The combination of both increased HR for MACE to 5.56; $p = 0.0002$. High MA-thrombin also predicted the risk for HPR (OR: 13.89; $p < 0.001$)
Gurbel et al. (114)	Patients undergoing PCI and taking aspirin and clopidogrel	225	36 months	ADP-induced (MA-ADP) and thrombin-induced (MA-thrombin) TEG measurement and LTA	Prediction of long-term event occurrence (ischemic and bleeding) following stenting	Patients with ischemic events had higher MA (ADP), MA (thrombin), and LTA ($p < 0.0001$ for all), which were independent predictors of ischemic events at 3 years (HR: 10.3, 3.8, and 4.8, respectively; all $p < 0.0001$)
Tang et al. (115)	Patients undergoing PCI divided into 3 groups depending on inhibition rates to aspirin and clopidogrel ($n = 90$): control group ($n = 30$) and resistance group ($n = 60$), who were then randomized to 2 subgroups ($R + R$ and $R + L$) to receive different antiplatelet combinations	90	12 months	TEG	Occurrence of CV ischemic events (including stent thrombosis, recurrent unstable angina, and MI)	Patients resistant to antiplatelet therapy vs. nonresistant control groups, had an increased risk of stent thrombosis (20% vs. 3%), recurrent unstable angina (36% vs.10%), and (MI 17% vs. 1%; $p < 0.01$). Randomization to a loading dose regimen improved inhibition rates and reduced the rates of CV events ($p < 0.01$)
Gurbel et al. (116)	Patients undergoing PCI	84	24 months	TEG and conventional aggregometry. Biomarker evaluation with fluorokine multianalyte profiling	Thrombogenicity and biomarkers of inflammation and correlation to the occurrence of ischemic events	Patients with high MA had an ischemic event more often than patients with low MA (48% vs. 13%; $p = 0.02$). Those in the highest MA group demonstrated higher levels of CRP, IL-8, and epidermal and vascular endothelial growth factors.
Gurbel et al. (117)	Patients undergoing nonemergent PCI	192	6 months	ADP-induced LTA and TEG	Platelet reactivity and clot strength and the risk of post-discharge ischemic events	Patients experiencing ischemic events ($n = 38$) demonstrated higher platelet reactivity by LTA ($63 \pm 12\%$ vs. $56 \pm 15\%$; $p = 0.02$), higher clot strength (MA) (74 ± 5 mm vs. 65 ± 4 mm; $p = 0.001$) and more rapid fibrin generation (4.3 ± 1.3 min vs. 5.9 ± 1.5 min; $p = 0.001$)

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TABLE 4 Continued

First Author (Ref. #)	Population	Patients (n)	Follow-Up	Method	Primary Endpoint	Results
Bliden et al. (118)	Patients receiving aspirin (325 mg qd) and clopidogrel (75 mg qd) undergoing nonemergent PCI	100	12 months	Measurement of platelet aggregation by standard LTA and TEG	Correlation between heightened platelet aggregation and occurrence of ischemic events	High on-treatment platelet reactivity, as measured by aggregometry and TEG, were significantly related to ischemic events ($p = 0.001$ for both assays).
Gurbel et al. (119)	African-American and Caucasian patients undergoing elective PCI	252	6 months	TEG	Assess race and sex difference in thrombogenicity and relate this to adverse ischemic events	TEG-derived platelet clot strength measurements (RR: 2.52; $p = 0.017$) and sex (RR: 2.56; $p = 0.009$) as independent predictors of ischemic events. African-American women exhibited higher thrombogenicity than the other race and sex groups ($p < 0.05$)
Kreutz et al. (24)	Patients with coronary artery disease, treated with aspirin and clopidogrel	211	3 ± 1.9 yrs	Platelet aggregometry assessed by LTA and clot formation using TEG. Genotyping of Val34Leu using TaqMan assay	Evaluate effects of Val34Leu on fibrin generation, platelet aggregation, and long-term clinical outcomes	Homozygous carriers of 34Leu variant had the greatest risk of MI and CV death ($p = 0.002$), associated with reduced fibrin clot formation time (TEG K: 1.27 ± 0.3 min vs. 1.68 ± 1.1 min; $p = 0.011$).
Tang et al. (120)	Chinese patients undergoing PCI for ACS	577	12 months	Detection of CYP2C19 G681A and $P2Y_{12}$ C34T polymorphisms by ligase detection reaction. Platelet reactivity assessed by TEG	Clopidogrel responsiveness and MACE (CV death, nonfatal MI, target vessel revascularization, and stent thrombosis)	118 patients with mutational A allele of CYP2C19 and mutational T allele of $P2Y_{12}$ demonstrated lowest ADP inhibition (49.74 ± 32.61%) and highest prevalence of clopidogrel low response (29.7%), which correlated with the highest CV event rates (8.5% vs. 1.5%).
Wu et al. (121)	NSTEMI patients undergoing PCI	233	24 h	CYP2C19*2 and *3 LOF alleles were evaluated using DNA microarray method. Platelet reactivity assessed by TEG	CYP2C19 genotype on HPR and risk of periprocedural MI	HPR more frequent in patients with periprocedural MI and an independent risk factor following multivariate analysis (OR: 4.348; $p = 0.001$). HPR also correlated with 2 CYP2C19 LOF allele carriage, associated with a 3-fold increased risk ($p = 0.037$).

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TABLE 4 Continued

First Author (Ref. #)	Population	Patients (n)	Follow-Up	Method	Primary Endpoint	Results
Cao J et al. (122)	Elderly men with CV disease receiving daily aspirin therapy (>75 mg)	304	1.8 yrs	Platelet aggregation measured by LTA and TEG	MACE (composite of death, MI, unstable angina, stroke, and transient ischemic attack)	Aspirin resistance (assessed by TEG) not associated with vascular events (17.7% vs. 10.9%; p = 0.452), although aspirin-resistance (defined by LTA) increased risk of composite outcome (18.3% vs. 9.8%; HR: 1.864; p = 0.003)
Tang et al. (123)	Chinese patients undergoing PCI	670	12 months	Antiplatelet effect assessed by TEG, CYP2C19, ABCB1, and PON1 genotypes detected by ligase detection reaction	Relationship between genotype variants on clopidogrel responsiveness and correlation to MACE (CV death, nonfatal MI, target vessel revascularization, and stent thrombosis)	CYP2C19 LOF alleles found in 57.3% of patients and associated with a gene dose-dependent effect on the risk of low response to clopidogrel and adverse ischemic events
Dridi et al. (124)	Patients with STEMI undergoing urgent PCI	233	12 months	Platelet activity measured with TEG-MA.	Relationship between TEG and myocardial damage (assessed with CMR) in STEMI patients	TEG-defined hypercoagulation present in 35.2% not correlated with infarct size, myocardial salvage index, or adverse events.

ABCB1 = ATP-binding cassette, sub-family B, member 1; ADP = adenosine diphosphate; CMR = cardiac magnetic resonance; CYP2C19 = cytochrome P450 2C19; DNA = deoxyribonucleic acid; HPR = high platelet reactivity; IL = interleukin; LOF = loss-of-function; LTA = light transmittance aggregometry; MA = maximum amplitude; NSTEMI = non-ST-segment elevation myocardial infarction; PCI = percutaneous coronary intervention; PON1 = serum paraoxonase 1; qd = daily; STEMI = ST-segment elevation myocardial infarction; TEG = thromboelastography; other abbreviations as in Tables 2 and 3.

Because the GTT assesses both thrombus formation and thrombus lysis in native blood, without external agonists and using high shear, it is arguably the most physiological assessment of global thrombotic status currently available.

This test has been studied in patient groups at high risk of cardiovascular thrombosis, with early results suggesting that it may be useful in predicting clinical outcomes (60,61). It has not been compared with other platelet function tests or TEG. However, the results would not be expected to correlate because of the different flows (high vs. low shear stress) and the use of native versus anticoagulated blood (**Table 1**).

ENDOGENOUS FIBRINOLYSIS: EVIDENCE FOR AN IMPORTANT ROLE IN CARDIOVASCULAR DISEASE

PLASMA MARKERS OF FIBRINOLYSIS IN CARDIOVASCULAR DISEASE. A number of studies have attempted to examine the relationship between plasma markers of fibrinolysis, signifying impaired fibrinolysis, and the occurrence of cardiovascular events.

Genetic polymorphisms in key enzyme regulators of fibrinolysis may increase susceptibility to thrombotic events (62). The 4G4G phenotype of the 4G/5G PAI-1 gene polymorphism was found to be an independent predictor of AMI (odds ratio: 2.7, p = 0.002) (63), and was observed more frequently in patients with a previous history of AMI than in those with stable angina. A review of the prospective studies undertaken between 1999 and 2009, encompassing some 45 studies and nearly 50,000 patients, demonstrates the conflicting results regarding the usefulness of these markers (31). Most of these were large epidemiological studies assessing thousands of patients. **Table 2** summarizes publications between 1999 and 2015 that utilized multivariate analysis in patient cohorts >1,000 to assess the predictive value of plasma fibrinolysis markers for adverse cardiovascular events.

In 1 of the larger prospective studies that evaluated t-PA levels in 3,582 women, there was a weak correlation between t-PA and the development of coronary artery disease (25). In patients with established coronary disease or AMI, t-PA levels were predictive of future cardiovascular events. In the Caerphilly Study of 2,398 men with 13 years of follow-up, baseline PAI-1 levels were significantly associated with cardiovascular risk (64), but after multivariate analysis, the correlation became nonsignificant (64). Other studies evaluating PAI-1 demonstrated a significant association with coronary events (65), cardiogenic shock,

death (32), and major adverse cardiovascular events (MACE) (32). In the Framingham study involving 3,209 participants, PAI-1 levels were not related to cardiovascular events (66). Other studies have also failed to demonstrate a prognostic role for baseline t-PA or PAI-1 levels (27,28). There is also conflicting data from studies on the role of other plasma markers of fibrinolysis, including D-dimer assays (25,28,31,64), plasmin-alpha2-antiplasmin complex measurements (31), TAFI (31), and Lp(a) levels (31,32).

Even allowing for publication bias, in that negative studies are less likely to be published, it is clear that biomarkers of fibrinolysis may, at best, allow a weak prediction of increased cardiovascular risk at a population level only. It is difficult to ascertain global fibrinolytic status on the basis of the plasma level of 1 or even several biomarkers. Furthermore, there is still controversy regarding the ideal laboratory technique to use. Determination of the total antigen levels of plasma markers can be achieved using enzyme-linked immunosorbent assays; alternatively, measurement of specific biological activity levels of plasma markers can be undertaken with immunofunctional chromogenic substrate kinetic assays. With some plasma markers, such as PAI-1, which has a relatively long half-life (approximately 1 h), there is likely to be a good correlation between PAI-1 antigen and PAI-1 activity levels. However, a poor correlation has been demonstrated between measurements of TAFI antigen and TAFI activity (67), which may be a reflection of its short half-life (approximately 10 min).

These problems with plasma marker measurements are confounded by the additional role of complementary pathways involved in mediating endogenous fibrinolysis. Studies have demonstrated the importance of plasma fibrin architecture in facilitating effective endogenous fibrinolysis (68) (**Central Illustration**). Additionally, the release of proteolytic enzymes from thrombus-associated neutrophils, namely elastase, has been shown to result in direct digestion of fibrin and inactivation of PAI-1 (69) (**Central Illustration**). Moreover, thrombus-adherent monocytes have been demonstrated to enhance TAFI activity, reducing fibrinolytic activity and protecting against clot lysis (70).

Platelets represent an important source of PAI-1, containing up to 90% of the total PAI-1 content of blood (71). During thrombus formation, activated platelets release high local concentrations of PAI-1, which serve to inhibit thrombolysis and stabilize clot formation (**Central Illustration**). The most functionally important source of PAI-1 is, therefore, platelets, and this pool of PAI-1 varies independently of plasma PAI-1 levels (72–74).

These studies have highlighted that regulation of thrombus formation is a dynamic, multifaceted phenomenon, and measurements of individual components of the pathway do not give an accurate reflection of this complex system.

GTT IN CARDIOVASCULAR DISEASE. Because the balance between prothrombotic factors and endogenous thrombolytic activity determines the propensity for thrombus formation in ACS, an overall assessment of thrombotic risk requires a global evaluation of a patient's thrombotic profile, including platelet reactivity, activation of the coagulation system (thrombin generation), and endogenous fibrinolysis.

Clinical studies evaluating the GTT are shown in **Table 3**. A study of 300 patients with ACS (61) revealed that although platelet reactivity was reduced, endogenous thrombolysis was impaired in ACS patients compared with healthy volunteers, despite taking dual antiplatelet medication. There was no correlation between OT and MACE. Some 23% of ACS patients had a markedly prolonged LT, a finding that was not demonstrated in normal subjects. Impaired endogenous thrombolysis was an independent predictor of MACE. LT >3,000 s was identified as the optimal cutoff point to predict MACE; above this level, the hazard ratio for cardiovascular events increased as the LT increased. LT remained a statistically-significant predictor for MACE, even after adjustments for a number of baseline cardiovascular risk factors.

The GTT has also been used to assess the thrombotic profile of patients with established cardiovascular risk factors. LT was significantly prolonged in smokers compared with nonsmokers, whereas no significant difference in OT was observed (75). There was a direct correlation between LT and daily cigarette consumption. Following 3 months of smoking cessation, LT values were found to be significantly shorter when compared with baseline GTT measurements. Another study demonstrated impaired endogenous thrombolytic activity in elderly male patients and in those who smoked, but showed no difference in OT (76), suggesting that the increased susceptibility of smokers to thrombosis may, in part, be related to decreased fibrinolytic activity. In patients with metabolic syndrome, LT was significantly prolonged compared with normal volunteers, and was associated with significantly higher PAI-1 levels, although no difference was observed in OT (77).

Patients with end-stage renal disease (ESRD) are at much higher cardiovascular risk than the general population (60,78), and impairment of endogenous fibrinolytic activity has also been observed, with reduced t-PA secretion and elevated levels of

fibrinogen and PAI-1 (79). Patients with ESRD demonstrated significantly prolonged OT and LT compared with normal volunteers (60). Additionally, 42% of patients demonstrated an LT >3,000 s and 34% demonstrated markedly impaired fibrinolytic status with LT >6,000 s, compared with none of the control subjects. LT was strongly predictive of the composite of cardiovascular death, nonfatal myocardial infarction, cerebrovascular events, and peripheral thrombotic events, even after adjustment for baseline variables. No relationship between OT and MACE was observed.

TEG IN CARDIOVASCULAR DISEASE. TEG has been applied to guide the use of blood and blood products during trauma resuscitation (80) and liver and cardiac surgery (81,82), and, more recently, it has also been evaluated in obstetric patients (83). It has now been reliably demonstrated that TEG detects hyperfibrinolysis in the perioperative and trauma setting (84,85), with increasing evidence that TEG-guided algorithms can help to optimize patient management (82,86). Its indications for use in the assessment of cardiovascular patients are ever expanding, including the monitoring of patients on aspirin, clopidogrel, and glycoprotein IIb/IIIa antagonists (87,88). A number of studies have demonstrated that TEG-derived measurements of platelet responsiveness can be utilized as a prognostic marker to predict the risk of long-term ischemic events (**Table 4**).

However, TEG has proven to be a less robust measure of hypofibrinolysis, with unmodified TEG assays in normal subjects exhibiting only a minor degree of fibrinolysis. Indeed, in 1 study, the normal range of ROTEM maximum lysis at 60 min was demonstrated to be <12% (range 0% to 12%) (89). The current limitation with existing TEG techniques to evaluate hypofibrinolysis has prompted the development of novel methods to improve its sensitivity. These techniques have included the use of exogenous urokinase or t-PA in concentrations that allow for the assessment of clot formation, whilst simultaneously enhancing clot lysis, permitting more accurate assessment of hypofibrinolysis (90). However, there has been no formal standardization and very little published data on these approaches, and further work is required to improve the sensitivity and standardization of TEG techniques to evaluate hypofibrinolysis.

A large number of studies have evaluated the usefulness of TEG in assessing clot strength. In this regard, TEG has been shown to be very useful in predicting increased cardiovascular risk in patients with established coronary disease and in those undergoing percutaneous coronary intervention (**Table 4**).

CONCLUSIONS

Although previously viewed as a secondary phenomenon in response to the formation of thrombi, a large body of evidence now points to a much more prominent role for endogenous fibrinolysis in thrombus formation.

The technical limitations, difficulty in interpretation, and conflicting data regarding prognostic usefulness of plasma markers in patients with coronary disease limit their adoption into clinical practice. Recognition of these limitations prompted the development of global assays of fibrinolytic status. TEG is a useful tool for assessing bleeding risk, and has also been used to assess clot strength, which has been shown to predict future cardiovascular events. However, its practical value in assessing the (spontaneous) thrombolytic status of patients or the effect of medications is questionable, due to its inability to assess the procoagulant and fibrinolysis-inhibiting properties of platelets and its low shear-stress milieu, which more closely resembles venous flow. The GTT provides a physiological assessment of global thrombotic status by assessing both thrombus formation and thrombus lysis in native blood in a high-shear setting that is relevant to arterial flow. Early clinical studies suggest that it may be useful in identifying patients at risk of future cardiovascular events. Endogenous fibrinolysis, until recently a poorly-understood area, represents an expanding and exciting area for identifying patients at increased cardiovascular risk and as a potential target for pharmacological modulation to improve outcomes.

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KEY WORDS atherosclerosis, platelet activation, platelet aggregation, platelet function tests, thrombolysis, thrombosis