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.

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).

From the *East & North Hertfordshire NHS Trust, Hertfordshire, United Kingdom; and yVascular Sciences, National Heart & Lung Institute, Imperial College, London, United Kingdom. Prof. Gorog is related to a company director of Thromboquest Ltd., who manufactures the Global Thrombosis Test, but she, her spouse, and her children have no financial involvement or equity interest in, and have received no financial assistance, support, or grants from Thromboquest Ltd. Thromboquest Ltd. has no involvement in the design, conduct, or the finance of this review. Dr. Okafor has reported that he has no relationships relevant to the contents of this paper to disclose.

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IMPACT 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, this pathway has been relatively over-

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 thrombolyis. 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; TxA₂ = thromboxane A₂.
3. The structure and stability of the resultant fibrin network is also a determinant of thromboreistance (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.
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<tr>
<th>First Author (Ref. #)</th>
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<th>Results</th>
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<tbody>
<tr>
<td>Kinlay et al. (28)</td>
<td>RCT</td>
<td>2,860</td>
<td>ACS patients enrolled in MIRACL study</td>
<td>16 weeks</td>
<td>CV events (death, nonfatal MI, cardiac arrest, or worsening angina)</td>
<td>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)</td>
</tr>
<tr>
<td>Zamani et al. (106)</td>
<td>RCT</td>
<td>2,925</td>
<td>ACS patients enrolled in MIRACL study</td>
<td>16 weeks</td>
<td>Death and recurrent nonfatal ACS (MI or unstable angina)</td>
<td>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).</td>
</tr>
<tr>
<td>Wang et al. (66)</td>
<td>Prospective</td>
<td>3,209</td>
<td>Subjects in 6th cycle of Framingham Offspring Study (1995–1998)</td>
<td>10 yrs</td>
<td>Death and major CV events (MI, unstable angina, heart failure, and stroke) and nonmajor CV events</td>
<td>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).</td>
</tr>
<tr>
<td>Folsom et al. (34)</td>
<td>Prospective</td>
<td>6,391</td>
<td>Subjects without atherosclerosis</td>
<td>4.6 yrs</td>
<td>Cancer death, mortality, and CAD (MI or coronary death), and CV disease (cardiac arrest, angina or revascularization and stroke)</td>
<td>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).</td>
</tr>
<tr>
<td>May et al. (25)</td>
<td>Prospective</td>
<td>3,582</td>
<td>Women without prior CAD</td>
<td>4.7 yrs</td>
<td>Development of CV death, MI, or coronary revascularization</td>
<td>D-dimer, t-PA antigen, and vWF were not associated with development of CAD after adjustment for CV risk factors.</td>
</tr>
<tr>
<td>Cushman et al. (108)</td>
<td>Nested case-control study</td>
<td>5,201 (146 selected cases)</td>
<td>Patients without baseline vascular disease</td>
<td>2.4 yrs</td>
<td>Coronary death, MI, and angina.</td>
<td>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.</td>
</tr>
<tr>
<td>Nordenhem et al. (107)</td>
<td>Case-control study</td>
<td>1,267</td>
<td>Patients with first MI identified</td>
<td>Matched to control group</td>
<td>MI</td>
<td>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).</td>
</tr>
<tr>
<td>Smith et al. (64)</td>
<td>Prospective</td>
<td>2,398</td>
<td>Men age 49–65 yrs</td>
<td>4 yrs</td>
<td>CV events (coronary heart disease and ischemic stroke combined)</td>
<td>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 VIIIc was inversely related to CV events (HR: 0.75; p = 0.001).</td>
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<tr>
<td>Morange et al. (109)</td>
<td>Prospective</td>
<td>1,057</td>
<td>Patients with CAD (AtheroGene Study)</td>
<td>6.6 yrs</td>
<td>CV death and nonfatal CV events (MI and stroke)</td>
<td>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). Total TAFI not associated with CV events.</td>
</tr>
<tr>
<td>Tregouet et al. (110)</td>
<td>Prospective</td>
<td>1,668</td>
<td>Patients with CAD (AtheroGene Study)</td>
<td>2.3 years</td>
<td>CV death and nonfatal CV event (MI)</td>
<td>Activated TAFI independently associated with risk of CV death (HR: 2.38; 95% CI: 1.56-3.63; p &lt; 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.</td>
</tr>
<tr>
<td>Gaw et al. (111)</td>
<td>Prospective</td>
<td>5,732</td>
<td>Elderly patients with risk factors for, or established vascular disease</td>
<td>3.2 yrs</td>
<td>CV death, nonfatal MI, fatal or nonfatal stroke</td>
<td>Lp(a) levels not associated with primary endpoint (HR: 1.05; 95% CI: 1.00-1.11; p = NS).</td>
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<tr>
<td>Bennet et al. (112)</td>
<td>Case-control study</td>
<td>2,047</td>
<td>Patients without CAD or stroke at baseline who experienced an MI or coronary death</td>
<td>NA</td>
<td>First-ever MI or coronary death</td>
<td>Lp(a) values in the top vs. bottom third, after multivariate adjustment for CV risk factors, OR: 1.60; 95% CI: 1.07-2.67 (p values not published). Progressive increase in OR with higher Lp(a) levels.</td>
</tr>
<tr>
<td>Willeit et al. (91)</td>
<td>Prospective case-control study</td>
<td>1,925</td>
<td>Patients without CV disease who experienced MI or coronary death</td>
<td>19.4 yrs</td>
<td>First-ever MI or coronary death</td>
<td>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 &lt; 0.001).</td>
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<tr>
<td>Wannamethee et al. (92)</td>
<td>Prospective study</td>
<td>3,217</td>
<td>Men age 60-79 yrs without CAD</td>
<td>7 yrs</td>
<td>Coronary death, nonfatal MI, and uncomplicated angina</td>
<td>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).</td>
</tr>
<tr>
<td>Chien et al. (93)</td>
<td>Prospective cohort study</td>
<td>3,484</td>
<td>Chinese patients without CAD</td>
<td>13.8 yrs</td>
<td>All-cause death, stroke, and CAD</td>
<td>Lp(a) levels did not correlate with risk of CV disease (HR: 0.81; p = NS).</td>
</tr>
<tr>
<td>Gurdasani et al. (94)</td>
<td>Prospective cohort study</td>
<td>18,720</td>
<td>Healthy subjects, age 39-79 yrs</td>
<td>11.4 yrs</td>
<td>Peripheral artery disease, stroke, and CAD-related events</td>
<td>Lp(a) levels associated with CAD hospitalization and mortality (HR: 1.13; p &lt; 0.00001).</td>
</tr>
<tr>
<td>Nestel et al. (95)</td>
<td>Prospective study</td>
<td>7,863</td>
<td>Patients with prior coronary event</td>
<td>6 yrs</td>
<td>Coronary death, nonfatal MI, ischemic stroke, revascularization, total CV events, and total coronary events</td>
<td>Lp(a) levels correlated with CV events (p &lt; 0.001), total CV events (HR: 1.33; p = 0.002), and coronary events (p = 0.03).</td>
</tr>
<tr>
<td>Kwon et al. (96)</td>
<td>Prospective</td>
<td>1,494</td>
<td>Type 2 diabetic patients with CAD</td>
<td>4.4 yrs</td>
<td>MACE (cardiac deaths and nonfatal MI)</td>
<td>Highest Lp(a) level tertile associated with MACE (HR: 2.89; p = 0.005).</td>
</tr>
<tr>
<td>Kwon et al. (97)</td>
<td>Prospective</td>
<td>6,252</td>
<td>Patients with suspected CAD</td>
<td>3.1 yrs</td>
<td>MACE (cardiac death and nonfatal MI)</td>
<td>Elevated Lp(a) associated with MACE (HR: 1.77; p = 0.005).</td>
</tr>
<tr>
<td>Canoui-Poitrine et al. (100)</td>
<td>Prospective cohort study</td>
<td>9,711</td>
<td>Men age 50-59 yrs free of CAD and stroke</td>
<td>10 yrs</td>
<td>CAD events (angina, MI, and coronary death) and ischemic stroke</td>
<td>Lp(a) levels associated with CV events (HR: 1.12; p = 0.001) after adjustment for risk factors.</td>
</tr>
<tr>
<td>Virani et al. (101)</td>
<td>Prospective</td>
<td>13,318 (n = 3,467 blacks, n = 9,851 Caucasians)</td>
<td>African-American and Caucasian adults without CHD or stroke</td>
<td>20 yrs</td>
<td>CV events (coronary death, MI, silent MI, revascularization) and stroke</td>
<td>Lp(a) levels associated with CV events. Quintile analysis for the highest compared with the lowest quintile demonstrated an HR: 1.35 (p = 0.004) for blacks and HR: 1.27 (p = 0.001) for whites.</td>
</tr>
<tr>
<td>O’Donoghue et al. (102)</td>
<td>Prospective</td>
<td>6,708</td>
<td>Patients with CAD from 3 studies (PEACE, CARE, and PROVE-IT-TIMI 22 trial)</td>
<td>MACE (composite of CV death, MI, or stroke)</td>
<td>No association between Lp(a) levels and MACE in any of the 3 trials individually or combined.</td>
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<td>Okafor and Gorog (1690)</td>
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**TABLE 2**

**First Author (Ref. #) Design Patients (n) Population Follow-Up Endpoint Results**

**Endogenous Thrombolysis in CV Disease**

APRIL 28, 2015:1683

J A C V O L .6 5 , N O .1 6 , 2 0 1 5

Okafor and Gorog

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<th>Endogenous Thrombolysis in CV Disease</th>
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**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 (¼ 95% CI: 1.04 – 1.08; p = 0.86) for CAD.

Shilpak et al. (105) RCT 2,763 Post-menopausal women age 4.1 yrs CV events (nonfatal MI and Lp(a) levels associated with CV events < 80 yrs with CAD CV death) (HR: 1.54; 95% CI: 0.99 – ¼ 0.03)

ACS¼ acute coronary syndromes; CAD¼ coronary artery disease; CARE¼ Cholesterol And Recurrent Events; CHD¼ coronary heart disease; CI¼ con
dence interval; CRP¼ C-reactive protein; CV¼ cardiovascular; HR¼ hazard ratio; Lp(a)¼ lipoprotein (a); MACE¼ major adverse cardiovascular events; MI¼ Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering; NA¼ not applicable; NS¼ not significant; OR¼ odds ratio; PAP¼ plasmin-alpha2-antiplasmin complex; PEACE¼ Prevention of Events With Angiotensin-Converting Enzyme Inhibitor Therapy; PROVE-IT-TIMI 22¼ randomized controlled trial; RR¼ relative risk; TAFI¼ thrombin-activatable fibrinolysis inhibitor; TAT¼ thrombin-antithrombin complex; vWF¼ von Willebrand factor; other abbreviations as in Table 1.

mitigates the contribution of platelet activation and situation in stenosed arteries. Furthermore, this venous stasis and does not re
take the low shear stress environment encountered with cardiac surgery, its practical value in assessing the (spontaneous) thrombo
dysfunction of patients or the effect of medications is questionable. The shortcomings of this technique begin with the testing of citrated and recalci
ced blood. The effect of extracellular calcium concentration on coagulation indexes and thromboelastography results is significant (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) thrombotic status of patients or the effect of medications is questionable. The shortcomings of this technique begin with the testing of citrated and recalci
ced 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 recalci
ced 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, non-anticoagulated 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**

<table>
<thead>
<tr>
<th>First Author (Ref. #)</th>
<th>Population</th>
<th>Patients (n)</th>
<th>Follow-Up</th>
<th>Methods</th>
<th>Primary Endpoint</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sharma et al. (60)</td>
<td>End-stage renal disease patients on hemodialysis</td>
<td>216</td>
<td>276 ± 166 days</td>
<td>GTT</td>
<td>MACE (CV death, nonfatal MI, CVA, and peripheral arterial thrombosis)</td>
<td>Impaired endogenous thrombolysis (LT &gt;3,000 s) strongly associated with MACE (HR: 4.25; p = 0.004), nonfatal MI, and CVA (HR: 14.28; p = 0.01) and peripheral thrombosis (HR: 9.08; p = 0.003)</td>
</tr>
<tr>
<td>Saraf et al. (61)</td>
<td>ACS patients receiving dual antplatelet therapy</td>
<td>300</td>
<td>12 months</td>
<td>GTT</td>
<td>MACE (CV death, nonfatal MI, or CVA)</td>
<td>LT &gt;3,000 s was an independent predictor of MACE (HR: 2.52; p = 0.004) and CV death (HR: 4.2; p = 0.033).</td>
</tr>
<tr>
<td>Saraf et al. (61)</td>
<td>ACS vs. healthy control subjects</td>
<td>300</td>
<td>N/A</td>
<td>GTT</td>
<td>MACE (CV death, nonfatal MI, or CVA)</td>
<td>OT prolonged in ACS (428 s vs. 378 s; p &lt; 0.001) and LT shorter in ACS (1,053 s vs. 1,362 s; p &lt; 0.001) than in control subjects</td>
</tr>
<tr>
<td>Suehiro et al. (75)</td>
<td>Healthy subjects of smoking and nonsmoking status</td>
<td>Smokers = 76 vs. nonsmokers = 63</td>
<td>3 months</td>
<td>GTT</td>
<td>Effect of smoking on thrombotic profile</td>
<td>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</td>
</tr>
<tr>
<td>Ikarugi et al. (76)</td>
<td>Healthy young males and elderly males</td>
<td>Young – 30 vs. elderly – 34</td>
<td>N/A</td>
<td>GTT</td>
<td>Effect of age, smoking, and exercise on thrombotic profile</td>
<td>LT was significantly longer in elderly vs. young (p &lt; 0.0001), and prolonged in elderly smokers than nonsmokers (p &lt; 0.0001)</td>
</tr>
<tr>
<td>Suehiro et al. (77)</td>
<td>Males with MetS vs. control subjects</td>
<td>MetS – 30 vs. control – 53</td>
<td>N/A</td>
<td>GTT</td>
<td>Comparison of thrombotic profile between groups</td>
<td>LT significantly longer in MetS than in control subjects (1,494 s vs. 1,246 s). PAR-1 level correlated with LT (p &lt; 0.001)</td>
</tr>
<tr>
<td>Rosser et al. (98)</td>
<td>ACS or stable coronary disease randomized to vorapaxar vs. placebo, in addition to standard of care</td>
<td>57</td>
<td>N/A</td>
<td>GTT</td>
<td>Thrombotic status, as shown by OT and LT of GTT</td>
<td>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)</td>
</tr>
<tr>
<td>Taomoto et al. (99)</td>
<td>Acute cerebrovascular disease (CVA) vs. healthy control subjects</td>
<td>CVA = 185 vs. control subjects = 195</td>
<td>N/A</td>
<td>GTT</td>
<td>Thrombotic status, as shown by OT and LT of GTT</td>
<td>In stroke patients, OT was shorter (p &lt; 0.0001) and LT was longer (p &lt; 0.0001) than in healthy control subjects</td>
</tr>
</tbody>
</table>

CVA = cerebrovascular accident (stroke); LT = lysis time; MetS = metabolic syndrome; OT = occlusion time; other abbreviations as in Tables 1 and 2.
<table>
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<tr>
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</thead>
<tbody>
<tr>
<td>Jeong et al. (113)</td>
<td>PCI-treated patients receiving aspirin and clopidogrel</td>
<td>197</td>
<td>24 months</td>
<td>MA-thrombin TEG measurements, conventional aggregometry, and genotyping</td>
<td>Relationship between MA-thrombin on high on-treatment platelet reactivity (HPR) and long-term MACE</td>
<td>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 &lt; 0.001)</td>
</tr>
<tr>
<td>Gurbel et al. (114)</td>
<td>Patients undergoing PCI and taking aspirin and clopidogrel</td>
<td>225</td>
<td>36 months</td>
<td>ADP-induced (MA-ADP) and thrombin-induced (MA-thrombin) TEG measurement and LTA</td>
<td>Prediction of long-term event occurrence (ischemic and bleeding) following stenting</td>
<td>Patients with ischemic events had higher MA (ADP), MA (thrombin), and LTA (p &lt; 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 &lt; 0.0001)</td>
</tr>
<tr>
<td>Tang et al. (115)</td>
<td>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</td>
<td>90</td>
<td>12 months</td>
<td>TEG</td>
<td>Occurrence of CV ischemic events (including stent thrombosis, recurrent unstable angina, and MI)</td>
<td>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 &lt; 0.01). Randomization to a loading dose regimen improved inhibition rates and reduced the rates of CV events (p &lt; 0.01)</td>
</tr>
<tr>
<td>Gurbel et al. (116)</td>
<td>Patients undergoing PCI</td>
<td>84</td>
<td>24 months</td>
<td>TEG and conventional aggregometry. Biomarker evaluation with fluorokine multianalyte profiling</td>
<td>Thrombogenicity and biomarkers of inflammation and correlation to the occurrence of ischemic events</td>
<td>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.</td>
</tr>
<tr>
<td>Gurbel et al. (117)</td>
<td>Patients undergoing nonemergent PCI</td>
<td>192</td>
<td>6 months</td>
<td>ADP-induced LTA and TEG</td>
<td>Platelet reactivity and clot strength and the risk of post-discharge ischemic events</td>
<td>Patients experiencing ischemic events (n = 38) demonstrated higher platelet reactivity by LTA (63 ± 12% vs. 56 ± 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)</td>
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</table>

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<table>
<thead>
<tr>
<th>First Author (Ref. #)</th>
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<tbody>
<tr>
<td>Blieden et al. (118)</td>
<td>Patients receiving aspirin (325 mg qd) and clopidogrel (75 mg qd) undergoing nonemergent PCI</td>
<td>100</td>
<td>12 months</td>
<td>Measurement of platelet aggregation by standard LTA and TEG</td>
<td>Correlation between heightened platelet aggregation and occurrence of ischemic events</td>
<td>High on-treatment platelet reactivity, as measured by aggregometry and TEG, were significantly related to ischemic events ( p = 0.001 ) for both assays.</td>
</tr>
<tr>
<td>Gurbel et al. (119)</td>
<td>African-American and Caucasian patients undergoing elective PCI</td>
<td>252</td>
<td>6 months</td>
<td>TEG</td>
<td>Assess race and sex difference in thrombogenicity and relate this to adverse ischemic events</td>
<td>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 &lt; 0.05 ).</td>
</tr>
<tr>
<td>Kreutz et al. (24)</td>
<td>Patients with coronary artery disease, treated with aspirin and clopidogrel</td>
<td>211</td>
<td>3 ± 1.9 yrs</td>
<td>Platelet aggregometry assessed by LTA and clot formation using TEG. Genotyping of Val34Leu using TaqMan assay</td>
<td>Evaluate effects of Val34Leu on fibrin generation, platelet aggregation, and long-term clinical outcomes</td>
<td>Homozygous carriers of 34Leu variant had the greatest risk of MI and CV death ( p = 0.002 ), associated with reduced fibrin clot formation time ( \text{TEG K: } 1.27 \pm 0.3 \text{ min vs. } 1.68 \pm 1.1 \text{ min; } p = 0.011 ).</td>
</tr>
<tr>
<td>Tang et al. (120)</td>
<td>Chinese patients undergoing PCI for ACS</td>
<td>577</td>
<td>12 months</td>
<td>Detection of CYP2C19 G681A and P2Y12 C34T polymorphisms by ligase detection reaction. Platelet reactivity assessed by TEG</td>
<td>Clopidogrel responsiveness and MACE (CV death, nonfatal MI, target vessel revascularization, and stent thrombosis)</td>
<td>118 patients with mutational A allele of CYP2C19 and mutational T allele of P2Y12 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%).</td>
</tr>
<tr>
<td>Wu et al. (121)</td>
<td>NSTEMI patients undergoing PCI</td>
<td>233</td>
<td>24 h</td>
<td>CYP2C19*2 and *3 LOF alleles were evaluated using DNA microarray method. Platelet reactivity assessed by TEG</td>
<td>CYP2C19 genotype on HPR and risk of periprocedural MI</td>
<td>HPR more frequent in patients with periprocedural MI and an independent risk factor following multivariate analysis ( \text{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 ).</td>
</tr>
</tbody>
</table>

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### Table 4 Continued

<table>
<thead>
<tr>
<th>First Author (Ref. #)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Okafor and Gorog</td>
<td>Elderly men with CV disease receiving daily aspirin therapy (&gt;75 mg)</td>
<td>304</td>
<td>1.8 yrs</td>
<td>Platelet aggregation measured by LTA and TEG</td>
<td>MACE (composite of death, MI, unstable angina, stroke, and transient ischemic attack)</td>
<td>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)</td>
</tr>
<tr>
<td>Cao J et al. (122)</td>
<td>Elderly men with CV disease</td>
<td>304</td>
<td>1.8 yrs</td>
<td>Platelet aggregation measured by LTA and TEG</td>
<td>MACE (composite of death, MI, unstable angina, stroke, and transient ischemic attack)</td>
<td>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</td>
</tr>
<tr>
<td>Tang et al. (123)</td>
<td>Chinese patients undergoing PCI</td>
<td>670</td>
<td>12 months</td>
<td>Antplatelet effect assessed by TEG, CYP2C19, ABCB1, and PON1 genotypes detected by ligase detection reaction</td>
<td>Relationship between genotype variants on clopidogrel responsiveness and correlation to MACE (CV death, nonfatal MI, target vessel revascularization, and stent thrombosis)</td>
<td>CYP2C19 genotype and ABCB1 genotypes were associated with a gene dose-dependent effect on the risk of low response to clopidogrel and adverse ischemic events</td>
</tr>
<tr>
<td>Dridi et al. (124)</td>
<td>Patients with STEMI undergoing urgent PCI</td>
<td>233</td>
<td>12 months</td>
<td>Platelet activity measured with TEG-MA.</td>
<td>Relationship between TEG and myocardial damage assessed with CMR in STEMI patients</td>
<td>TEG-defined hypercoagulation present in 35.2% not correlated with infarct size, myocardial salvage index, or adverse events</td>
</tr>
</tbody>
</table>

**Abbreviations:**
- APRIL 28, 2015:1683
- TEG = Thromboelastography
- ABCB1 = ATP-binding cassette, sub-family B, member 1
- ADP = Adenosine diphosphate
- O2R = Oxygenation
- CVR = Coronary vascular resistance
- BM = Bone marrow
- LOF = Loss-of-function
- ST-segment elevation myocardial infarction
- PCI = Percutaneous coronary intervention
- MI = Myocardial infarction
- TEG = Thromboelastography
- MACE = Composite of death, MI, stroke, and transient ischemic attack
- LTA = Light transmittance aggregometry
- TEG = Thromboelastography
- PON1 = Serum paraoxonase 1
- ABCB1 = ATP-binding cassette, sub-family B, member 1
- CYP2C19 = Cytochrome P450 2C19
- DNA = Deoxyribonucleic acid
- MI = Myocardial infarction
- HPR = High platelet reactivity
- IL = Interleukin
- ADP = Adenosine diphosphate
- CMR = Cardiac magnetic resonance
- TEG = Thromboelastography
- other abbreviations as in Tables 2 and 3.
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

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**Endogenous Thrombolysis in CV Disease**

Okafor and Gorog

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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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Endogenous Thrombolysis in CV Disease

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