The pivotal role of hypoactive endogenous fibrinolysis in the occurrence of thrombotic cardiovascular events is now well-recognized. To evaluate the diagnostic and prognostic role of impaired fibrinolysis, plasma fibrinolysis markers have been investigated in large prospective studies in both healthy individuals and patients with established coronary disease. Antigen and activity levels of components of the fibrinolytic system were measured by immunoassays, which replaced earlier global fibrinolysis tests. This review covers 45 studies in nearly 50,000 subjects, examining the association between plasma markers of fibrinolysis and coronary artery disease, to establish the usefulness of these markers in predicting future cardiovascular events. The predictive value of plasma levels of tissue-type plasminogen activator, platelet activator inhibitor-1, plasmin-antiplasmin complex, D-dimer, thrombin activatable fibrinolysis inhibitor, and lipoprotein(a) for major adverse cardiac events is highly variable and conflicting, especially after adjusting for conventional risk factors, judging from the published data in the last decade. The value of fibrinolysis activity markers is very limited in aiding diagnosis and risk stratification in the individual patient, on the basis of the weak prognostic values obtained in some studies and the lack of power in others. The physiological limitations of such markers in reflecting endogenous fibrinolysis is discussed. The emerging novel global assays of fibrinolysis will require large-scale clinical trials before their prognostic power or superiority to multiple biomarker measurements can be evaluated. (J Am Coll Cardiol 2010; 55:2701–9) © 2010 by the American College of Cardiology Foundation

Scope of This Review

The measurement of plasma markers of fibrinolysis dates back more than 20 years. This review covers the last 10 years, from 1999 to July 2009. Earlier findings were reviewed by Lijnen and Collen (1) and Hoffmeister et al. (2). A new review is timely, because a number of new fibrinolysis markers have been discovered and a large number of clinical studies have been carried out with improved, more sensitive immunoassays.

This review covers 45 prospective studies in nearly 50,000 subjects, examining the association between plasma markers of fibrinolysis and coronary artery disease (CAD), to establish the usefulness of these markers in predicting future cardiovascular events. Of the prospective studies, only those that have evaluated the results by multivariate regression analysis and calculated relative risk are shown in Table 1.

Significance of Endogenous Fibrinolysis in Acute Coronary Syndromes (ACS)

Endogenous fibrinolysis is a protective mechanism against lasting arterial thrombotic occlusion, which would otherwise lead to permanent tissue damage. Because arterial thrombogenesis is an active, ongoing, and dynamic process, a healthy endogenous fibrinolytic system can prevent the build-up of thrombus before complete occlusion occurs or break up the occlusive thrombus before lasting tissue damage ensues.

Until the early 1980s, coronary artery spasm and diminished myocardial oxygen supply were regarded as the fundamental mechanisms of myocardial infarction (3,4). Coronary artery thrombosis as the major pathogenic mechanism of sudden death from ischemic heart disease was disputed mainly because autopsy studies reported a very low incidence of identifiable coronary thrombus. By performing early autopsies, with improved techniques, the British pathologist Dr. Michael Davies first reported coronary thrombi in 74 of 100 subjects who died of ischemic heart disease and, of all these, only 5 subjects had no acute arterial lesion (5). Similar findings were reported by others, showing that the infarct-related artery was occluded in 90% of cases within the first 6 h of acute myocardial infarction (AMI), whereas this fell to 57% by 12 to 24 h (6). Growing evidence from cases of spontaneous lysis of arterial thrombi further supported the pathology findings and provided an explanation for the failure of earlier studies to detect thrombi at late autopsies (7–10).

Over the last decade, increasing evidence has emerged to support the assumption that AMI is a failure of timely
spontaneous thrombolysis (11). Spontaneous reperfusion of the infarct-related artery was reported to occur frequently in patients with AMI and was associated with significant myocardial salvage (12). In 585 patients with ST-segment elevation myocardial infarction (STEMI), electrocardiographic or angiographic spontaneous reperfusion (SR) was observed in 15%, and those with SR had lower mortality, lower composite of death/shock/congestive heart failure, and significant reduction in death or reinfarction (13). In a recent study of 710 patients with STEMI undergoing primary percutaneous coronary intervention (PCI), SR was observed in 22% of patients and at 30 days was associated with significantly lower duration of death, congestive heart failure, and recurrent ACS (14). It has been suggested that impaired fibrinolysis at the time of coronary angioplasty contributes to restenosis (15). Deficient local endothelial tissue-type plasminogen activator (TPA) release in patients with CAD indicates reduced local fibrinolytic capacity and might explain the increased risk of coronary thrombosis in this patient group (16). Recently, low plasma fibrinolytic potential, found in 10% of the population, was found to increase the relative risk of arterial thrombosis 2-fold (17).

Assessment of Overall Fibrinolytic Status by Biomarkers

The mechanism and elements of the fibrinolytic system have been reviewed recently, and are shown in simplified schematic in Figure 1 (18,19). Thrombin converts the inactive proenzyme plasminogen to active plasmin. Plasmin degrades the cross-linked fibrin into soluble degradation products by the tissue-type (TPA) and the urokinase type plasminogen activators. It is TPA that is mainly responsible for the dissolution of fibrin formed in the circulation. This fibrinolytic system can be inhibited either by antagonizing plasmin through alpha 2-antiplasmin or by specific plasminogen activator inhibitors (PAI). There are 3 types of PAI described so far; of these, physiologically the most important inhibitor is PAI type 1 (PAI-1). The thrombin activatable fibrinolysis inhibitor (TAFI) is another important

### Table 1

<table>
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<tr>
<th>D-Dimer</th>
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<th>PAP</th>
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<th>TAFI-Ag</th>
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**Abbreviations and Acronyms**

- ACS = acute coronary syndrome
- AMI = acute myocardial infarction
- CAD = coronary artery disease
- ELISA = enzyme-linked immunosorbent assay
- HR = hazard ratio
- Lp(a) = lipoprotein(a)
- MACE = major adverse cardiovascular event
- OR = odds ratio
- PAI = plasminogen activator inhibitor
- PAP = plasmin-alpha2-antiplasmin complex
- PCI = percutaneous coronary intervention
- SR = spontaneous reperfusion
- STEMI = ST-segment elevation myocardial infarction
- TAFI = thrombin activatable fibrinolysis inhibitor
- TAFI-Ag = thrombin activatable fibrinolysis inhibitor antigen
- TPA = tissue-type plasminogen activator

**Studies Using Multivariate Analysis to Demonstrate Either NS or Significant Predictive Value of Plasma Fibrinolysis Marker Levels for MACE**

<table>
<thead>
<tr>
<th>Markers</th>
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</table>

Numbers are presented as n with the number of the relevant reference shown in parentheses. Significances (where given in the citation): *p < 0.05; †p < 0.02; ‡p < 0.001; ‡‡p < 0.001. MACE = major adverse cardiovascular event; NS = not significant; PAI = plasminogen activator inhibitor; PAP = plasmin-alpha2-antiplasmin complex; TPA = tissue-type plasminogen activator.
inhibitor of the fibrinolytic system. The TAFI forms a link between blood coagulation and fibrinolysis. Thrombin forms fibrin to stabilize the platelet-rich thrombus and at the same time produces TAFI to protect that fibrin network. The TAFI circulates as an inactive proenzyme in blood and becomes activated by thrombin during blood clotting. The active form (TAFIα) inhibits fibrinolysis by cleaving off C-terminal lysine residues from partially degraded fibrin that stimulate the TPA-mediated conversion of plasminogen to plasmin. Consequently, removal of these lysines leads to less plasmin formation and subsequently to protection of the fibrin clot from breakdown.

Although the plasmin-mediated fibrinolytic system is the major physiological mechanism of removing fibrin from the circulation, the plasmin-independent breakdown of fibrin by cellular components of blood also plays a significant role. An arterial thrombus contains significant amounts of leukocytes (neutrophils and monocytes) covering the thrombus surface or incorporated into it, due to interaction of activated platelets with leukocytes. Neutrophil membrane proteolytic enzymes (elastase, cathepsin G) can break down fibrin directly and can assist the dissolution of thrombus by plasmin (20). Furthermore, the physical properties of fibrin formed during hemostasis/thrombosis greatly affect the rate and effectiveness of fibrinolysis. Clots formed from blood of ACS patients are composed of dense networks of fibrin that are more resistant to lysis than clots derived from patients with stable angina (21).

In the past decade, clinical studies on endogenous fibrinolysis have investigated the following biomarkers: alpha2-antiplasmin-plasmin complex; alpha-2 antiplasmin, TPA, markers of fibrin turnover, such as fibrin degradation products, D-dimer, and soluble fibrin. Discovery of TAFI and the finding that plasma lipoprotein(a) [Lp(a)], a homologue of plasminogen, can inhibit TPA-mediated plasminogen activation on fibrin surfaces have attracted much attention, and the contribution of these 2 physiologically important fibrinolysis inhibitors to overall fibrinolytic status has also been investigated in patients with CAD.

**TPA and PAI-1**

Paradoxically, increased plasma TPA indicates inhibited endogenous fibrinolysis. This is because free TPA released into blood from endothelial cells immediately forms a complex with circulating PAI-1. Thus assays of TPA antigen measure mainly inactive TPA/PAI-1 complexes. Concentration of the TPA/PAI-1 complex levels in plasma correlated strongly with levels of TPA antigen and PAI-1 activity (22,23).

There is evidence that TPA levels predict cardiovascular disease in apparently healthy individuals. In a prospective study of 3,582 women ages 60 to 70 years, there was a positive but weak association (hazard ratio [HR]: 1.2) between TPA and the development of CAD (24). In a nested case-control study of CAD patients and healthy individuals, TPA was an independent risk factor for the evolution of coronary disease (25). Elevated TPA antigen was independently associated with incident coronary events (odds ratio [OR]: 3.5) in post-menopausal women (26).

Furthermore, TPA levels predict future cardiovascular events in patients with established CAD. In stable angina patients (27) and in a number of studies involving AMI patients, including the MIRACL (Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering) study of 2,860 patients, raised TPA level was related to greatly increased risk of recurrent events (28–30).

The PAI-1 levels have also been related to adverse cardiovascular outcomes in both healthy individuals and cardiac patients. The Caerphilly Study of 2,398 middle-aged men followed-up for 13 years found that baseline levels of PAI-1 but not TPA were significantly associated with the
occurrence of a cardiovascular event (31). A prospective study of 249 angina patients found that those with higher PAI-1 activity (especially diabetic patients) had a 4.2-fold risk of subsequent coronary events (32). In 180 AMI patients, raised levels of PAI-1 on admission were associated with greatly increased risk of cardiogenic shock and in-hospital and 1-year mortality (OR: 6.0) (33). In 520 patients with ACS, increased PAI-1 was a significant and independent risk factor for the occurrence of major adverse cardiovascular events (MACE) (OR: 5.3) (34). A recent case control study found that the 4G4G genotype of the 4G/5G PAI-1 gene polymorphism was more frequently observed in patients with prior AMI than those with stable coronary disease and was an independent predictor of AMI (OR: 2.7, p = 0.002) (35).

Similarly, levels of TPA/PAI-1 complex also predict future events. In the SHEEP (Stockholm Heart Epidemiology Program) study of some 800 AMI patients and matched control subjects, high levels of TPA/PAI-1 complex were significantly associated with AMI risk, with a synergistic interaction observed in men for the co-exposure to high plasma TPA/PAI-1 complex concentrations and smoking, diabetes mellitus, and serum cholesterol (36).

However, there have also been negative studies. In the 3,209 participants of the Framingham study, PAI-1 levels were not related to cardiovascular events (37). In the ADVANCE (Action in Diabetes and Vascular Disease: Preterax and Diamicron Modified Release [MR] Controlled Evaluation) study, plasma TPA and PAI-1 levels did not distinguish patients presenting with AMI from those with stable angina (38). Furthermore, 2 small prospective studies in AMI patients followed for 3 years, which also did not identify a prognostic role for baseline TPA or PAI-1 levels in predicting future cardiovascular events (39,40).

D-Dimer

There are mixed data on the predictive value of plasma D-dimer level in healthy individuals. In large, prospective studies of elderly subjects, D-dimer level has been shown to predict future AMI (OR: 2.5) (41) and cardiovascular death (OR: 4.0) (42). In the Caerphilly Study, where 2,398 middle-aged men were followed-up for 13 years, D-dimer level was associated with future cardiovascular events (31). In post-menopausal women, D-dimer was independently associated with the occurrence of coronary events (OR: 2.0) (26). In contrast, in 3,209 participants in the Framingham study, plasma D-dimer was not predictive of MACE (37).

Another large prospective study of 3,582 women ages 60 to 70 years found no association between D-dimer and development of CAD (24). In the Multiethnic Study of Atherosclerosis, raised D-dimer levels were related to increased risk of death but not nonfatal cardiovascular events in more than 6,000 subjects (43).

In patients with established CAD, data are again conflicting. In the MIRACL study of 2,860 patients with ACS (28) as well as 2 other smaller studies in ACS (39,44), D-dimer levels were not predictive of subsequent cardiovascular events. However, in AMI patients undergoing primary PCI (n = 102), baseline D-dimer was significantly higher in patients who developed subsequent restenosis (61%) than those who did not (25%) (45). Furthermore, in patients with decompensated heart failure, D-dimer levels were significant predictors of mortality (46).

The specificity of D-dimer for cardiovascular disease is questionable. In 257 patients with chest pain, raised D-dimer had an independent diagnostic value for AMI (positive and negative predictive values estimated as 92% and 41%, respectively) and increased the diagnostic sensitivity of the electrocardiogram and history from 73% to 92% (47). In another study of 279 patients with suspected ACS, D-dimer showed significantly higher positive test rate for the detection of ACS in the very early phase compared with troponin T and therefore was regarded useful for the screening of ACS in the emergency setting (48). In the ADVANCE Study, however, D-dimer did not distinguish patients presenting with AMI from those with stable angina (38). In large prospective studies investigating a multivariate approach to the initial assessment of patients with chest pain, D-dimer did not aid detection of ACS or predict prognosis above and beyond established risk factors (49,50).

PAP

There are conflicting data on the value of plasmin-alpha2-antiplasmin complex (PAP) measurement. In the Cardiovascular Health Study of 5,201 healthy men and women ages >65 years, PAP level predicted future AMI (OR: 3.1) (41). In 200 AMI patients, low plasma PAP level was associated with subsequent coronary events (OR: 5.0) (51). In the large Multiethnic Study of Atherosclerosis (n = 6,391), PAP was associated with increased risk of death (HR: 2.00) but not with nonfatal cardiovascular events (43). In contrast, however, in the AtheroGene study of 1,057 CAD patients, PAP was not predictive of subsequent cardiovascular death (52), and in a small study of patients undergoing elective PCI, PAP failed to differentiate between patients with and those without restenosis (53).

TAFI

High TAFI plasma levels might contribute to a hypo fibrinolytic state and to an increased risk of thrombotic episodes.

Some studies reported low thrombin activatable fibrinolysis inhibitor antigen (TAFI-Ag) levels in patients with CAD and attributed a cardio-protective role to reduced TAFI levels against MACE, contrary to expectation. In a case-control study of some 600 AMI patients, TAFI-Ag value above the 90th percentile was associated with a significantly lower risk of AMI (OR: 0.55) (54). Similarly, in the case-control SMILE (Study of Myocardial Infarctions Leiden) of 554 men with a first AMI, low activated
TAFI levels were associated with increased risk of AMI (OR: 2.4 to 3.4) (55). The ATTAC (Role of Thrombin Activatable Fibrinolysis Inhibitor in Arterial Thrombosis at a Young Age) study identified the TAFI 325Thr/Ile polymorphism to be associated with lower TAFI levels and with increased risk of cardiovascular disease in young patients compared with the common 325Thr/Thr genotype (56). Polymorphism of the TAFI gene (Thr325Ile) was not only related to TAFI-Ag plasma levels but also to angiographic restenosis after PCI (57).

However, other studies have found CAD associated with raised TAFI levels. The AtheroGene Study in 1,668 patients with angiographically proven CAD showed that increase of activated TAFI, measured by TAFIa/TAFIai enzyme-linked immunosorbent assay (ELISA), but not of the total amount of TAFI to be independently associated with risk of cardiovascular death (HR: 1.7) (58). The TAFI levels also tended to be higher in CAD patients as compared with control subjects, especially in intracoronary blood samples (59). Furthermore, TAFI-Ag levels were found to be lower but functional activated TAFI levels were significantly higher in young patients with AMI than in control subjects (60). In patients with stable angina undergoing PCI, pre-procedural plasma TAFI-Ag levels were significantly higher in those who developed restenosis (61).

There were other studies that were neutral. The TAFI levels were not associated with an increased risk of arterial thrombosis in thrombophilic families (n = 1,940) (62). In 44 patients admitted to the coronary care unit and compared with 44 control subjects, no differences were found in either TAFI-Ag or activated TAFI between patients and control subjects (63).

**Lp(a)**

Early studies reported only a weak association of Lp(a) with thrombotic risk. In 520 ACS patients, Lp(a) was not a risk factor for future MACE (34). The larger PROSPER (prospective study of pravastatin in the elderly at risk) Study Group, where entry Lp(a) levels were measured in 5,732 elderly subjects who were followed for 3.2 years, found a weak association between Lp(a) and risk of cardiovascular events (HR: 1.1, p = 0.032) (64).

Subsequent studies have found plasma Lp(a) levels to be consistently predictive of arterial thrombotic occlusion. In 144 patients with STEMI undergoing early coronary angiography (<12 h), baseline Lp(a) levels were inversely related to infarct-related artery patency, with highest Lp(a) levels associated with angiographic TIMI flow grade 0 to 1 (OR: 3.1) (65). In patients with premature coronary disease (n = 142), plasma Lp(a) levels were significantly higher than in control subjects (39). In the THROMBO (Thrombogenic Factors and Recurrent Coronary Events) study of obese post-infarction patients (n = 663), elevated Lp(a) was a highly significant risk marker of recurrent cardiovascular events (HR: 3.94) (66). In 876 subjects undergoing screening for vascular disease prevention, Lp(a) levels were strongly associated with the presence of silent carotid occlusion (OR: 1.7; p < 0.0001) (67).

However, the association between plasma Lp(a) levels and burden of coronary atheroma is less well-established. In 897 patients undergoing coronary angiography, Lp(a) was significantly associated with the presence and severity of coronary stenosis (OR: 1.2) (68). In 397 patients undergoing coronary angiography, high Lp(a) levels were independently associated with the presence of coronary obstruction (OR: 2.5) (69). Yet, in patients undergoing PCI with angiographic follow-up, plasma Lp(a) concentrations were not predictive of restenosis (70).

Recently the first meta-analysis of long-term prospective studies investigating Lp(a) published over the last 40 years, involving nearly 127,000 subjects, was reported. Under a wide range of circumstances, there were independent and modest associations of Lp(a) concentration with risk of coronary disease (HR: 1.13, 95% confidence interval: 1.1 to 1.2) and stroke that seem exclusive to vascular outcomes (71).

A recent study found only the oxidized Lp(a) to be associated with presence and severity of ACS and suggested that oxidized Lp(a) plasma level measurement could be useful for identification of patients with ACS (72).

Despite the growing interest in the predictive power of this fibrinolysis marker, existing methodological problems still prevent the use of Lp(a) as a routine diagnostic tool (73). However, existing evidence shows that Lp(a) levels are related to cardiovascular risk, which is mediated through thrombosis and impaired fibrinolysis.

**Prognostic and Diagnostic Value of Fibrinolysis Markers**

The overall outcome of these studies for all measured markers is highly controversial. Even without allowing for publication bias, the number of negative studies unable to demonstrate an association or predictive power is practically equal to those reporting a positive association and prognostic value. Even some of the so-called “positive” studies report relative risks that, although significant, are so weak (HR: 1.1 to 1.7) that they indicate only a statistical trend but have little practical usefulness. Conflicting findings of either low or high TAFI levels being associated with cardiovascular risk might be related to the discrepancy between measurements of total TAFI-Ag or TAFI activity, either in all patients or in only those with specific TAFI genotypes. Furthermore, methodological problems curtail the use of Lp(a) in routine clinical practice.

The limitations of these biomarkers (see the following text) might provide an explanation for the conflicting results and for the failure of large-scale studies of fibrinolysis markers to reflect the true endogenous fibrinolytic status in CAD patients.
Limitations of Fibrinolysis Markers

1. Do not reflect leukocyte-mediated fibrinolysis; neutrophil elastase interferes with D-dimer and PAI-1 measurements. As mentioned earlier, leukocyte-derived enzymes, mainly elastase, released from thrombus-associated neutrophils, effect fibrinolysis by direct digestion of fibrin or indirectly modulate it by degradation inhibitors of the fibrinolytic system. Neutrophil elastase degrades fibrin and also degrades D-dimer and inactivates PAI-1 (74). Although the cleavage and inactivation of PAI-1 by neutrophil elastase enhances fibrinolysis, degradation reduces plasma D-dimer concentration and might be partly responsible for discrepant results when comparing different D-dimer assays. Fibrinogen elastase degradation products might serve as a specific marker of elastase proteolytic activity. In a case-control study, significantly higher fibrinogen elastase degradation product levels were observed in patients with ischemic stroke than in control subjects, both in the acute and in the convalescent phase (75).

2. Plasma PAI-1/TAFI measurements do not reflect endogenous thrombolytic capacity. The main limitation of plasma PAI-1 measurement is that it does not reflect the local release of PAI-1 from activated platelets during thrombogenesis. Platelets contain approximately 90% of the total amount of PAI-1 present in blood (76). During thrombus formation, thrombin is generated by activated platelets resulting in the release of functionally active PAI-1 from platelet granules. The resultant high local concentration of PAI-1 in the thrombus mass prevents thrombolysis. It has been shown that the plasma and the platelet pools of PAI-1 vary independently of each other in disease states (77). Platelet-derived PAI-1 concentration was much higher than plasma PAI-1 on day 1 after AMI, and clearance rates of plasma and platelet PAI-1 from the systemic circulation were different (78). Thus, measurement of plasma PAI-1 level bears very little relation to the true contribution of PAI-1 (mainly from platelet release) to the stability of an arterial thrombus. That PAI-1 is predominantly platelet derived, is demonstrated by a recent report that clopidogrel pre-treatment completely abolishes the increase in PAI-1 active antigen after coronary stenting (79). Peri-procedural platelet activation might play a major role in the increase in PAI-1 after PCI, increasing the risk of acute and subacute thrombus formation not only through platelet activation but also due to impairment of the endogenous fibrinolytic system. By inhibiting platelet P2Y(12) receptors, which are involved in thrombin generation, clopidogrel inhibits thrombin-induced release of PAI-1 from platelets.

In addition to PAI-1 release, TAFI activation is also associated with thrombogenesis. The TAFI is activated through proteolytic cleavage by thrombin. Thrombin does not form in the systemic circulation but is generated by activated platelets during thrombus formation. Thrombin not only plays a central role in thrombogenesis by forming a fibrin network that confers structural stability to the labile platelet aggregate but is also involved in stabilizing the formed thrombus by activating TAFI. Activated TAFI protects the fibrin-stabilized thrombus against lysis (80). Recent findings show that thrombus-adherent monocytes inhibit fibrinolysis through a tissue factor-mediated enhancement of TAFI activation and increase the resistance of the thrombus to fibrinolysis (81). Because both thrombin generation and TAFI activation are highly localized processes in the thrombus, plasma TAFI level measurements bear little relevance to the actual thrombolytic status.

3. Measurements of total antigen, specific activities, or gene polymorphism of fibrinolysis markers yield conflicting clinical results. It is still not clear whether total antigen or activity measurements better reflect the physiological situation. Both quantitative and qualitative differences exist between studies measuring either the total antigen levels by the standard commercial ELISA methods and those reporting biological activities by immunofunctional chromogenic substrate kinetic assays. In plasma from patients with coronary disease, no statistical difference in PAI-1 activity, PAI-1 antigen, or PAI-1/TPA complex was observed compared with normal subjects (82). Because of the relatively long plasma half-life of PAI-1 activity (approximately 1 h), antigen and activity measurements might go hand in hand in healthy subjects. However, the poor correlation between measurements of TAFI-Ag and activity might be because activated TAFI is quickly inactivated by a rapid spontaneous conversion to the latent form with a half-life of approximately 10 min (83). Therefore, the very short half-life of TAFIa presents a practical measurement problem, which is difficult to overcome in clinical settings (84). This might explain why 1 study found that only the plasma level of activated TAFI, measured by TAFIa/TAFlai ELISA but not the total amount of TAFI was independently associated with risk of cardiovascular death (56,58).

Because background plasma PAI-1 and TAFI concentrations are almost entirely genetically determined, the controversial clinical findings might be explained by gene variations, including polymorphisms in the PAI-1 and TAFI genes. Compared with the commonest haplotype of the PAI-1 gene G-5G-A-A-T (35.1%), the haplotype A-4G-A-A-C (32.7%) significantly increased the risk of coronary disease and showed significant associations with common risk factors (85,86). By contrast, a randomized study found no impact of the PAI-1 4G/5G polymorphism on the amount of myocardial salvage achieved by reperfusion with stenting or thrombolysis in patients with AMI (87). Moreover, although the 4G allele was associated with higher PAI-1 levels, there was no association of the PAI-1 gene polymorphism with the risk of AMI (88,89).

4. Do not reflect hypofibrinolysis caused by altered fibrin architecture and the effects of drugs on fibrin network porosity. Physical properties of fibrin are major determinants of the efficacy of endogenous fibrinolysis. Abnormal plasma fibrin architecture seems to be an important feature
in thrombus formed in vitro from blood of young AMI survivors and impacts on fibrinolysis rate (90). Aspirin increases fibrinolysis, as demonstrated by shortened fibrin clot “lysis time” in parallel with an increase in fibrin network porosity (91). The effects of such altered fibrin architecture and drugs are not reflected in current biomarker assays.

**Biomarkers or Global Assays of Fibrinolysis?**

Even before the development of molecular markers and their immunoassays, global spontaneous fibrinolysis of human plasma had been measured. However, spontaneous fibrinolysis of plasma clots is an extremely slow process. In the quantification of clot lysis with the release of $^{125}$I from labeled fibrin, lysis was 10% or less at 20 h (92). Laboratory assays of global fibrinolysis such as the fibrin plate method and euglobulin clot lysis time greatly contributed to the understanding of the molecular mechanism of the fibrinolytic system but had little place in clinical practice, because they were labor-intensive and time-consuming. Recognition of the limitations of biomarkers in assessing global fibrinolytic status stimulated development of new global tests. The global thrombosis test measures the lysis of an autologous platelet-rich thrombus, formed from native blood exposed to high shear stress (93). The clot formation and lysis assay is an automated microtiter plate spectrophotometric assay of clot formation and lysis due to added TPA (94). The global assay of overall hemostasis potential measures clot-lysis by added rTPA, expressing the results as fibrinolysis profile (95). The fibrinolysis parameters assay is a rapid global assay for plasma fibrinolysis, whereas cellular fibrinolysis is measured by testing the clot-lysis capacity with polymorphonuclear neutrophils (96). The intrinsic oxidative clot lysis assay uses chloramines as the source of singlet oxygen to enhance urokinase type plasminogen activator-mediated lysis of plasma clots (97). The global fibrinolytic capacity of whole blood samples represents a global assessment of the main fibrinolytic factors in plasma and those associated with blood cells (98). The clot lysis time assay measures fibrinolytic activity by the lysis time of a tissue factor-induced clot (99).

This review shows that, despite improved specificity and refinement of factorial assays, the predictive value of fibrinolysis markers for coronary events is disappointing. This is at least in part due to the uncertainty about what to measure (antigens, activity, or genetic polymorphism in key proteins of the fibrinolytic system) as well as the inherent limitation of using a simple factorial assessment to quantify the complex and dynamic process of fibrinolysis. On a practical level, such individual markers fail to provide the rapid, meaningful, and individualized assessment of fibrinolytic status that is needed. From a clinical perspective, what is needed is a simple, global, point-of-care test that reflects not only overall plasma fibrinolytic capacity but also other determinants of endogenous thrombolytic activity, such as the contribution of platelets and the physical properties of the formed fibrin. The value of such global tests of fibrinolysis in the clinical setting and their superiority over the factorial approach with biomarkers remains to be established with well-conducted prospective studies.

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**REFERENCES**


34. Marcucci R, Brogi D, Sofi F, et al. PAI-1 and homocysteine, but not lipoprotein (a) and thrombophilic polymorphisms, are independently associated with the occurrence of major adverse cardiac events after successful coronary stenting. Heart 2006;92:377–81.


