Fibrinogen Assays for Cardiovascular Risk Assessment
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In this issue of Clinical Chemistry, Lovely and colleagues (1) report an evaluation of a new plasma fibrinogen assay for the study of its associations with cardiovascular disease. So, what is the current status of plasma fibrinogen in cardiovascular risk assessment, and how might this new assay advance our knowledge and practice?

Fibrinogen, the precursor of fibrin, was the first blood coagulation factor to be described—in 1836 by Buchanan at the University of Glasgow (2). By 1995, many studies had established that the plasma fibrinogen concentration was associated not only with coronary heart disease but also with cerebrovascular and peripheral vascular diseases (2). These associations have led to evaluation of fibrinogen as a possible causal factor in cardiovascular disease, as a therapeutic target, and as a risk predictor in both healthy persons and those with established cardiovascular diseases.

The association of fibrinogen with risks of cardiovascular disease and mortality in healthy individuals has been reliably established through international collaborative individual-person metaanalyses, the Fibrinogen Studies Collaboration (http://www.phpc.cam.ac.uk/MEU/FSC/), and its successor, the Emerging Risk Factors Collaboration (http://www.phpc.cam.ac.uk/MEU/ERFC/) (3, 4). The strength of fibrinogen’s associations with coronary heart disease and stroke is similar to those of classic risk factors, such as blood pressure and serum cholesterol, and to the circulating concentration of C-reactive protein (CRP), which, like fibrinogen, is a marker of the inflammatory response, including low-grade inflammation in healthy persons (4).

Is fibrinogen a causal risk factor in cardiovascular disease? Increasing fibrinogen concentrations might promote risk via effects on atherogenesis (like lipoproteins, fibrinogen infiltrates the arterial wall and can be converted to fibrin and its atherogenic degradation products), thrombogenesis (promoting both platelet aggregation and fibrin formation, and making thrombi more resistant to lysis), and ischemia (increasing plasma and whole-blood viscosity) (2, 5). Randomized controlled trials of acute reduction in plasma fibrinogen with a defibrinating snake venom enzyme, ancrod, have shown reductions in the risk for venous thromboembolism in surgical and stroke patients and a reduction in cerebral infarct size in acute ischemic stroke (6); however, the benefit of the latter effect was outweighed by an increased risk of intracranial bleeding.

Although the results of these trials may provide proof of principle that a decrease in fibrinogen reduces thrombosis and ischemia, establishing causality will require randomized trials of agents that selectively (and safely) reduce plasma fibrinogen over the long term. Such agents are not currently available. Certain fibrates reduce plasma fibrinogen concentrations by about 10%, but trials of fibrates cannot be used to test causality for fibrinogen concentrations because fibrates also alter the concentrations of lipids, lipoproteins, and homocysteine. Mendelian-randomized studies of genotypes associated with lifelong variation in fibrinogen concentrations have not shown associations with cardiovascular risk to date (3); however, known genotypes explain only 1% of the interindividual variation in plasma fibrinogen, limiting their power as causality tests.

Alternatively, the association of fibrinogen with cardiovascular risk may simply be as a marker of inflammation. Other inflammatory markers (CRP, white blood cell count, plasma viscosity, and erythrocyte sedimentation rate) show similar associations with cardiovascular risk and mortality. This fact likely reflects proximal effects of proinflammatory cytokines, such as interleukin-6 (7), on the hepatic synthesis of fibrinogen and CRP and on leukocyte release from bone marrow, as well as the effects of fibrinogen on plasma viscosity and red cell aggregation (5). Like CRP, fibrinogen shows similar associations with nonvascular mortality and vascular mortality (3, 4), and this nonspecificity for vascular disease raises questions regarding causality for such factors. Randomized controlled trials of agents reducing inflammation are required to assess its causality (8).

How should fibrinogen be measured in cardiovascular risk assessment? Hematology laboratories have traditionally measured fibrinogen by functional assays of thrombin-clottable protein. These assays are recommended for detecting hypofibrinogenemia or dysfibrinogenemia and for evaluating the risk of bleeding (9).
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Assays of clottable fibrinogen, however, require a citrated plasma sample, and clinical chemistry laboratories may also use immunologic or precipitation assays (of routine plasma samples containing potassium EDTA) for quantification of high fibrinogen concentrations (9). The Fibrinogen Studies Collaboration observed no significant differences between these 3 types of assays for associations of plasma fibrinogen with cardiovascular risk (3), and the available international standards include those suitable for all assay types (10).

Plasma fibrinogen, however, is a heterogeneous mixture of many different molecular forms (11), and different assays produce results that show limited agreement among healthy persons (12). Ratios of clottable fibrinogen to the “intact” fibrinogen synthesized by the liver are associated with cardiovascular risk, an observation that raises the possibility that the functionality of fibrinogen may be related to the risk of thrombosis (13). As reviewed by Lovely et al. (1), one determinant of the clottability of fibrinogen—and the lysability of fibrin—is the γ’ isoform of fibrinogen (14). Hence, assay of this isoform merits evaluation for risk assessment in cardiovascular disease.

Lovely et al. (1) describe and evaluate an immunometric ELISA assay for γ’ fibrinogen that showed acceptable precision. They studied 2879 healthy individuals from the Framingham Offspring Study to define the population reference interval and the associations with cardiovascular risk factors. Similarly to standard fibrinogen assays, the reference interval was wide (0.09–0.55 g/L), and γ’ fibrinogen concentrations were associated with most conventional risk factors. In contrast to standard plasma fibrinogen assays, however, γ’ fibrinogen showed no significant associations with blood pressure or the total cholesterol concentration. The authors therefore suggest that γ’ fibrinogen is not simply a surrogate marker for the fibrinogen assessed in standard fibrinogen assays.

Lovely et al. (1) also extended previous reports of the association of γ’ fibrinogen with cardiovascular disease by comparing patients with angiographic coronary artery disease with control individuals. Patients had significantly higher γ’ fibrinogen concentrations than controls, with a c statistic for the ROC curve of 0.76.

This new assay has potential to advance our understanding of the roles of fibrinogen in cardiovascular disease. Experimental and observational studies of γ’ fibrinogen concentrations (as well as their genetic determinants) compared with standard fibrinogen concentrations should help elucidate the functional role of fibrinogen in atherogenesis, thrombogenesis, and ischemia (2, 11, 13). Further large prospective studies of γ’ fibrinogen and standard fibrinogen assays should also be performed to establish their potential utility in adding to the currently used risk-prediction scores. In a recent large study, fibrinogen (assayed by a conventional thrombin-time assay) added significantly to the Framingham cardiovascular risk score in women, but not in men (15). In that study, fibrinogen did not add significantly to a risk score currently used in the UK, which, in addition to classic Framingham risk factors, includes a measure of socioeconomic status and family history of premature cardiovascular disease (15). For risk prediction, emerging laboratory risk predictors such as fibrinogen and CRP concentrations will eventually be judged on what they add to clinical risk scores that include emerging clinical risk factors.

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