Fibrin D-Dimer and Cardiovascular Risk

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ABSTRACT

Fibrin D-dimer, the most commonly used clinical assay for detection of coagulation activation and in vivo fibrin formation and lysis in circulating blood, has been associated with risks of cardiovascular diseases in studies published over the past 15 years. This review discusses, in turn, analytic and preanalytic considerations; associations with risk factors; and associations with coronary heart disease, atrial fibrillation, stroke and cerebrovascular disease, peripheral arterial disease, and venous thromboembolism. These associations suggest that activated coagulation and in vivo fibrin formation and lysis may play a role in arterial, intracardiac, and venous thromboembolism. The potential clinical utility of D-dimer in prediction of cardiovascular risk, in indicating patient groups for prophylactic anticoagulation and in monitoring of anticoagulation, requires further study. Harmonization of results from different assays would increase clinical utility.

KEYWORDS: Coagulation, fibrinolysis, fibrin degradation products, thrombosis, coronary heart disease, atrial fibrillation, stroke, peripheral arterial disease, epidemiology.

Educational Objectives: Upon completion of the article, the reader should (1) be able to describe associations of D-dimer with cardiovascular risk factors; and (2) recognize associations of D-dimer with risk of cardiovascular diseases.

Fibrin D-dimer is the most commonly used clinical assay for detection of coagulation activation and in vivo fibrin formation and lysis in circulating blood. Following the introduction of commercial, quantitative enzyme-linked immunoassays (ELISAs) in the 1980s,¹ D-dimer became well established in clinical practice for diagnosis of clinically suspected disseminated intravascular coagulation³ and for excluding the diagnosis of clinically suspected venous thromboembolism (VTE) by levels within the reference range⁴ (see also other articles in this issue).

Since the late 1980s, we have studied the relationships of D-dimer with cardiovascular risk, starting with the risks of adverse outcome following acute stroke.⁵ In 1992, we published the first epidemiological study of D-dimer, from the Glasgow MONICA survey⁶ (Fig. 1), showing that D-dimer was quantifiable in all healthy adults, with a positively skewed distribution, and an upper 95% confidence interval of 200 ng/mL, using the original AGEN ELISA (Parsippany, NJ).² By comparison, local patients with extensive clinical arterial disease (symptomatic peripheral arterial disease) had higher median levels of D-dimer, which correlated with clinical severity—increasing from intermittent claudication, through critical limb ischemia, to aortic aneurysm⁶ (Fig. 1). We therefore suggested that D-dimer might be a useful marker of cardiovascular disease and risk (arterial as well as venous) in the general population.
population, being a marker of intravascular fibrin formation.6–10 Such fibrin formation may be soluble (D-dimer levels correlate well with soluble fibrin levels)11,12 or released from digestion of subclinical or clinical intravascular thrombi. However, it should be stressed that circulating D-dimer levels are also increased in patients with extensive extravascular fibrin formation, such as those with rheumatoid arthritis,13,14 inflammatory bowel disease, renal failure treated by hemodialysis, and several cancers.8 Hence interpretation of D-dimer levels requires clinical appraisal of comorbidity in the individual subject.

Following our hypothesis that D-dimer might be a useful marker of cardiovascular risk,6 we showed in 1993 that D-dimer predicted both coronary heart disease (CHD) events and progression of peripheral atherosclerosis (fall in ankle-brachial pressure index, ABPI), in a 1-year follow-up of 617 patients with newly diagnosed intermittent claudication, consistent with the Rokitansky-Duguid hypothesis that fibrin formation contributes to progression of both coronary and peripheral atherothrombosis.7 We have subsequently confirmed that D-dimer was associated with risk of CHD in a meta-analysis of prospective studies15; we have also reported associations of D-dimer with risks of stroke,16 vascular dementia,17 adverse outcome after vascular surgery,18,19 and venous thromboembolism.20 This review of D-dimer and cardiovascular risk deals with each of these clinical areas in turn and starts with a review of analytic and preanalytic considerations and of the associations of D-dimer with cardiovascular risk factors.

**ANALYTIC AND PREANALYTIC CONSIDERATIONS**

**Assay Type and Source**

D-dimer is present in plasma across a wide range of fibrin derivatives of different molecular weights,11 and commercial assays vary widely in their absolute values and units (given as nanogram per milliliter, microgram per milliliter, or fibrin equivalents per milliliter).21,22 Such variation is mainly due to differences in antibody specificity (especially preference for high- or low-molecular weight fibrin derivatives) but also reflects...
differences in specificity for cross-linked versus non-cross-linked derivatives of fibrin(ogen) and other variables.\textsuperscript{21,22} If D-dimer is to be used in clinical practice for stratification of cardiovascular risk, it will be important to harmonize available assays across the population range, as well as in the “high-normal” range.

The epidemiological studies reviewed below have usually employed quantitative ELISAs to assay D-dimer. In our own laboratory, we initially used the original AGEN Dimertest ELISA.\textsuperscript{6,7,15–20} This was replaced by the AGEN “Gold” ELISA, which used a more specific signal antibody, resulting in much lower absolute levels compared with the original Dimertest ELISA.\textsuperscript{23} However, when directly compared in the Caerphilly Study, these two assays (which correlated fairly well, \(r = 0.59\)) showed similar associations with both CHD risk\textsuperscript{24,25} and CHD risk factors.\textsuperscript{26,27} The Behring Enzygnost ELISA (Marburg, Germany) also correlates well with the AGEN Dimertest ELISA \((r = 0.78)\) and again gives lower values.\textsuperscript{28} With the increasing use of rapid automated ELISAs and latex-based assays in routine clinical practice, the clinical utility of D-dimer in cardiovascular risk prediction may be enhanced by using such assays. For example, comparison of an ELISA and an automated latex agglutination test showed that they correlated well and yielded similar results in a study of prediction of recurrent myocardial infarction.\textsuperscript{29,30}

\section*{Plasma Versus Serum}

The epidemiological studies reviewed below have usually employed stored plasma (anticoagulated with tripotassium citrate, \(K_2\) ethylenediamine-tetracetic acid, or heparin) for D-dimer assays. We used long-stored serum (-20°C for 20 years) in a nested case-control study of CHD risk from the British Regional Heart Study, observing similar results to other prospective studies that used plasma stored for a shorter time.\textsuperscript{15} Although we observed a good correlation between plasma and serum in a prospective comparison,\textsuperscript{15} results were often quite different for serum and plasma in a comparative study of various D-dimer assays.\textsuperscript{22}

\section*{Sample Handling and Storage}

Delay in centrifuging citrated whole blood for 24 and 48 hours, at room temperature or at 4°C, did not significantly affect D-dimer levels; nor did storage for 1 year in a prospective study.\textsuperscript{31} As noted above, the association of D-dimer with CHD risk did not appear to vary by sample storage times.\textsuperscript{15} In a multicenter study, plasma separated within 6 hours of collection and stored for -20 to -80°C until airfreighting to a central laboratory showed satisfactory results.\textsuperscript{32}

\section*{Variability}

Within the population reference range, the interassay coefficient of variation of D-dimer in ELISA assay is 5 to 15\%.\textsuperscript{31,33} Biological intraindividual variation over 4 to 5 years appears similar to other cardiovascular risk factors.\textsuperscript{15,34} In the Edinburgh Artery Study,\textsuperscript{16} repeat measurements 5 years after baseline measurement in 1009 persons who had not experienced a cardiovascular event yielded a self-correlation coefficient of 0.51; these data were used to estimate the magnitude of regression dilution and to correct for it in a meta-analysis of CHD risk in prospective studies.\textsuperscript{15} More recently, repeat measurements 4 years after prior measurement in 297 men in the Dewsbury and Maidstone towns from the British Regional Heart Study showed a regression dilution ratio of 0.79 (95% confidence interval [CI]: 0.66, 091; MacMahon’s method).\textsuperscript{34} For comparison, in the same study regression dilution ratios for other risk factors were: high-density lipoprotein (HDL) cholesterol 0.87, total: HDL cholesterol 0.79, homocysteine 0.76, von Willebrand factor (vWF) 0.73, factor VII 0.72, total cholesterol 0.71, tissue plasminogen activator (tPA) antigen 0.71, triglyceride 0.65, fibrinogen 0.60, C-reactive protein 0.60, systolic blood pressure 0.59, glucose 0.59, and diastolic blood pressure 0.50.\textsuperscript{34}

\section*{ASSOCIATIONS WITH CARDIOVASCULAR RISK FACTORS}

\subsection*{Heritability}

In a recent twin study\textsuperscript{15} D-dimer showed significant heritability (65%), as did other markers of activated coagulation: prothrombin fragment F1 + 2 (F1 + 2) and thrombin-antithrombin complexes (TAT). We have recently confirmed significant heritability of D-dimer (75%) in another twin study.\textsuperscript{36} Further studies are required to identify the genetic basis of this heritable variation in activation of coagulation and fibrin formation.

\subsection*{Geography}

In a recent substudy of the World Health Organization MONICA project,\textsuperscript{32} we measured several thrombotic and inflammatory markers in men and women aged 45 to 64 years in 12 MONICA populations from 10 countries. All samples were collected using a carefully standardized protocol and assayed centrally. Following adjustment for age, smoking habit, and body mass index, geometric mean levels of D-dimer correlated significantly with coronary event rates in men and women across MONICA centers (Fig. 2). We observed similar findings for fibrinogen and vWF and concluded that these thrombotic risk factors might help explain differences in coronary risk between European populations.\textsuperscript{32}
Epidemiological studies have consistently found that D-dimer levels increase with age in men and women. In the Glasgow MONICA Survey, median levels of D-dimer (AGEN Dimentest ELISA) increased from 42 to 77 ng/mL over the age range 25 to 34 to 55 to 64 years among men; and from 51 to 75 ng/mL in women (P < 0.001 for both sexes). As noted above, UK levels may be higher than levels in other European countries with lower CHD risk.

**Age**

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**Sex, Hormone Use, and Pregnancy**

Likewise, epidemiological studies have consistently observed that D-dimer levels are higher in women than men. Possible reasons include higher fibrinogen levels in women than men; D-dimer is associated with fibrinogen in several studies with correlation coefficients of ~0.3. Alternatively, estrogens increase levels of coagulation activation and D-dimer, as shown in studies of users of combined oral contraceptives or hormone replacement therapy. D-dimer levels are increased in pregnancy and its complications (see review elsewhere in this issue).

**Smoking Habit**

Epidemiological studies have consistently observed that D-dimer levels are higher in current smokers with the exception of the...
control group of the British Regional Heart Study nested case-control study.15

**Physical Activity, Obesity, Insulin Resistance, and Diabetes**
Several epidemiological studies have observed an inverse correlation between regular physical activity and D-dimer.26,27,37,53 In the 20-year follow-up of the British Regional Heart Study, this inverse correlation was observed in both men with and men without prevalent cardiovascular disease.53 An inverse correlation between body mass index was also observed in the Edinburgh Artery Study37 but not in the Caerphilly Study.26,27 In the 20-year follow-up of the British Regional Heart Study, D-dimer showed modest age-adjusted correlations with body mass index (r = 0.06), waist circumference (r = 0.08), and insulin resistance (r = 0.04); however, these associations were not significant after adjustment for age, smoking, physical activity, alcohol intake, social class, and use of statins and aspirin.54 D-dimer was not associated with type 2 diabetes in this study.55

**Blood Pressure**
Most epidemiological studies have not observed significant correlations of D-dimer with either systolic or diastolic blood pressure.15,48,54,55 Reports of increased D-dimer levels in studies of patients with hypertension56–60 may therefore reflect their associations with vascular or renal complications of hypertension.

**Lipids and Lipoproteins**
Again, most epidemiological studies have not observed significant correlations of D-dimer with total, low-density lipoprotein, or HDL cholesterol; triglycerides; or apolipoproteins.15,48,54,55

**Alcohol**
In the British Regional Heart Study, weak inverse relationships of D-dimer were observed with alcohol consumption.61

**Use of Medication**
The effect of combined oral contraceptives and oral hormone replacement therapy on D-dimer levels has already been noted. D-dimer levels were also higher in middle-aged men using medication,26,27 most likely reflecting effects of underlying diseases. Warfarin lowers D-dimer levels in patients with venous thromboembolism,4 atrial fibrillation,62–64 left ventricular aneurysm,65 or increased risk of coronary heart disease.66

**Other Cardiovascular Risk Factors**
D-dimer levels did not correlate with socioeconomic factors, insulin, homocysteine, albumin, hematocrit, markers of infection (antibodies to *Helicobacter pylori* or *Chlamydia pneumoniae*), white cell count, or platelet count.15,40–42,48 D-dimer levels correlated with levels of the proinflammatory cytokine, interleukin-6 (IL-6),31,48 possibly due to a direct effect of D-dimer and other fibrin degradation products (FDPs) on IL-6 synthesis and release by monocytes in experimental studies.40–42,67 D-dimer levels also correlate with plasma levels of acute-phase reactant proteins, including C-reactive protein, serum amyloid A, fibrinogen, and also viscosity.15,40–42,48

**ASSOCIATIONS WITH CHD**

**Associations with Incident CHD Events**
In a meta-analysis of prospective studies of D-dimer and cardiovascular risk published before 2000,15 five population-based studies15,16,24,25,43,44 were identified; as were two studies in patients with baseline evidence of stable cardiovascular disease (CHD or claudication).7,29,30 These studies included a total of 1535 incident CHD events, all adjusted for age, smoking, blood pressure, and cholesterol, and there was no significant heterogeneity among them.15 A combined analysis yielded an odds ratio of 1.7 (95% CI: 1.3 to 2.2) in persons with baseline D-dimer values in the top third versus those in the bottom third, which corresponded to mean usual D-dimer values of 126 versus 50 ng/mL, respectively (AGEN Dimertest assay).15 (Fig. 3).

Other prospective studies40–42,68–70 have shown similar associations of D-dimer with incident CHD. A further meta-analysis in progress, including the Reykjavik Study with ~2500 CHD events,71 should clarify this association, as would a future collaborative meta-analysis of individual data, following the example of the Fibrinogen Studies Collaboration.72 Such an analysis would enable adjustment not only for cardiovascular risk factors but also for fibrinogen and other inflammatory markers15,40–42,48 and would also establish the incremental value of D-dimer for prediction of CHD risk over that provided by current CHD prediction scores (e.g., the Framingham risk score).

A potential causal role for hypercoagulability in CHD is suggested by the finding from the Thrombosis Prevention Trial that low-dose warfarin reduced CHD risk in high-risk men, but only at a sufficient intensity to lower D-dimer.66

**Associations with Prevalent CHD**
D-dimer was higher in persons with previous myocardial infarction in a case-control study.73 In the British
Regional Heart Study, levels of D-dimer (and other hemostatic variables) were increased in men aged 60 to 79 years with previous myocardial infarction, but not in men with uncomplicated stable angina. In contrast, D-dimer was increased (after adjustment for covariates) in patients with stable angina pectoris (and angiographically proved coronary artery disease) in a case-control study. Other epidemiological studies have observed increased D-dimer levels in subjects with baseline CHD compared with those without.

**Associations with Prognosis and Treatment in Acute Coronary Syndromes**

D-dimer levels are increased in patients with unstable angina or acute myocardial infarction. There is some evidence that levels are related to infarct size and risk of complications. D-dimer levels rise after thrombolytic therapy but are not associated with coronary artery patency or prognosis, and only a fraction of the elevation in D-dimer can be due to lysis of a coronary thrombus. At present, there is no proven value for routine assay of D-dimer in acute coronary syndromes, including thrombolytic therapy for acute myocardial infarction.

**Associations with Coronary Angioplasty**

D-dimer levels are increased following coronary angioplasty.

**Associations with Left Ventricular Dysfunction, Aneurysms, and Heart Failure**

Following myocardial infarction, left ventricular dysfunction, aneurysms, and heart failure may develop: each of these conditions is associated with increased D-dimer levels. Other epidemiological studies have observed increased D-dimer levels in subjects with baseline CHD compared with those without.

**ASSOCIATIONS WITH ATRIAL FIBRILLATION AND INTRACARDIAC THROMBOSIS**

Chronic atrial fibrillation (AF) is associated with increased risk of stroke and with increased D-dimer levels, irrespective of the presence of underlying structural heart disease or ventricular dysfunction. Patients with paroxysmal AF have intermediate levels of D-dimer, between those of patients with chronic permanent AF and persons in sinus rhythm. D-dimer levels normalize following cardioversion or full-dose warfarin but not after aspirin or minidose warfarin. These findings are consistent with the prophylactic efficacy of warfarin in AF.

Patients with AF and left atrial thrombus have higher levels of D-dimer than patients without complications. Following our suggestion that D-dimer be evaluated prospectively for risk of cardiovascular
complications in patients with AF, two prospective studies have reported associations of baseline D-dimer with cardiovascular complications (especially stroke), both in patients receiving warfarin and patients not receiving warfarin. Baseline D-dimer was also associated with decline in cognitive function in one of these studies, although warfarin had a beneficial effect. The potential use of D-dimer in selection of high-risk patients with AF for warfarin, or for tailored dosage of warfarin, requires further study.

ASSOCIATIONS WITH STROKE AND CEREBROVASCULAR DISEASE

Associations with Risk of Stroke and Dementia
As noted above, D-dimer in persons with atrial fibrillation was associated with risks of stroke and cognitive decline. In the general population, D-dimer was associated with risk of stroke in the Edinburgh Artery Study. D-dimer was also associated with dementia in a case-control study.

Associations with Diagnosis and Prognosis in Acute Stroke
As in acute coronary syndromes, D-dimer levels are raised in patients with acute stroke or transient cerebral ischemic attacks. Some studies have reported higher levels in cardioembolic or atherothrombotic stroke, compared with lacunar stroke. Three studies have reported that increased D-dimer levels were associated with adverse outcome or progression of ischemic stroke, and one did not. Two studies associated fragment E (another FDP) with adverse outcome or progression. Baseline FDP levels were associated with late clinical deterioration in the NINDS trial of thrombolytic therapy with t-PA. In a prospective study, median D-dimer levels were significantly higher in patients who developed proximal deep venous thrombosis (DVT), detected by screening with magnetic resonance imaging. A single measure of stroke severity at admission, and a D-dimer level at day 9 identified a substantial proportion of patients with proximal DVT. The potential use of D-dimer in selection of patients with acute stroke at high risk of progression, for anticoagulation requires further study.

ASSOCIATIONS WITH PREVALENT CEREBROVASCULAR DISEASE

Case-control and cross-sectional epidemiological studies have reported that D-dimer levels are elevated in patients with previous stroke. D-dimer levels were also associated with carotid atherosclerosis (intima-media thickness) in a cross-sectional study. In nested case-control studies from the PROGRESS study of blood pressure reduction following stroke, D-dimer was not associated with risk of recurrent stroke, whether ischemic or hemorrhagic. Few patients in this study had atrial fibrillation, in which two other studies have reported that D-dimer is a strong predictor of stroke.

ASSOCIATIONS WITH PERIPHERAL ARTERIAL DISEASE

Associations with Prevalent Peripheral Arterial Disease
Case-control and cross-sectional epidemiological studies have reported that D-dimer levels are elevated in patients with peripheral arterial disease and are correlated with clinical, hemodynamic, and angiographic severity. The highest levels are observed in patients with abdominal aortic aneurysms, in whom fibrin deposition and turnover within the aneurysmal sac are high.

Associations with Prognosis in Peripheral Arterial Disease
In a prospective study of 617 patients with newly diagnosed claudication, baseline D-dimer levels were associated at 1 year with both hemodynamic progression of peripheral arterial disease (fall in ABPI) and risk of CHD events. As noted in the introduction, these associations are consistent with the Rokitansky-Duguid hypothesis, that fibrin formation contributes to progression of both coronary and peripheral atherothrombosis. More prolonged follow-up of this cohort showed that D-dimer continued to be a predictor of CHD events, consistent with a meta-analysis of all prospective studies of CHD events.

D-dimer levels increase following percutaneous angioplasty, and baseline levels were associated with risk of early rethrombosis (within 30 days) following percutaneous transluminal angioplasty for peripheral arterial disease in one study, but not with restenosis after 2 to 3 years in another study. Baseline D-dimer levels have also been associated with adverse outcome following infrarenal bypass grafting and risk of perioperative myocardial infarction.

ASSOCIATIONS WITH VTE

As noted in the introduction, D-dimer levels have an established place in diagnosis of clinically suspected venous thromboembolism (extensively reviewed in other
Associations with Prevalent VTE
Two case-control studies\(^{20,134}\) have shown that patients with previous VTE have increased D-dimer levels, when studied at least 6 months after the acute event, following discontinuation of anticoagulant therapy. In one study, the association was not independent of thrombophilias,\(^{20}\) but the other study showed a supra-additive increase in the risk associated with the two common genetic thrombophilias, factor V Leiden and the pro-thrombin 20210A variant.\(^{134}\)

Associations with Incident VTE
In the European Concerted Action against Thrombosis (ECAT) multicenter study of clinical and laboratory predictors of postoperative DVT,\(^{28}\) D-dimer (measured by two different ELISAs in two laboratories) was associated with risk of postoperative DVT, detected by routine venography 10 to 14 days after elective hip replacement, on univariate analysis; this is consistent with the results of previous studies of baseline levels of FDP and postoperative DVT.\(^{135}\) However, D-dimer was associated with other risk factors for DVT (age, obesity, varicose veins) and was not a significant predictor of DVT after adjustment for these clinical variables.\(^{29}\)

In the Lifelong Incidence of Thromboembolic Events (LITE) prospective study, D-dimer was significantly associated with risk of VTE on multivariate analysis.\(^{46}\)

D-dimer levels have also been associated with risk of recurrent DVT, when measured at the end of a conventional course of oral anticoagulants, or following their discontinuation\(^{136,137}\) (see also review elsewhere in this issue).

CONCLUSION
The evidence reviewed suggests that activated coagulation and in vivo fibrin formation and lysis may play a role in arterial, intracardiac, and venous thromboembolism. Although D-dimer correlates with other activation markers (F1 + 2, TAT)\(^{25,28,47,73}\) and with soluble fibrin,\(^{12}\) it appears to be a stronger predictor of cardiovascular events, possibly due to its longer half-life and relative stability—both during sample taking and handling—and on long-term storage. Its biological variability appears comparable to other cardiovascular risk factors,\(^{34}\) and among new potential predictors of cardiovascular risk, it appears to be less influenced by conventional risk factors.\(^{15}\) However, its potential clinical utility as a risk predictor requires further study. In addition, harmonization of results from different assays would increase clinical utility.

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